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Turkish Journal of Biochemistry

XXVII. BALKAN KLİNİK LABORATUVAR FEDERASYONU KONGRESİ BCLF 2019

XXVII. BALKAN CLINICAL LABORATORY FEDERATION MEETING BCLF 2019

XXX. TÜRK BİYOKİMYA DERNEĞİ ULUSAL BİYOKİMYA KONGRESİ TBD 2019

27-31 Ekim 2019, Antalya, Türkiye

XXX. NATIONAL CONGRESS OF THE TURKISH BIOCHEMICAL SOCIETY TBS 2019

27-31 October 2019, Antalya, Turkey

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BCLF WELCOME LETTER

Dear friends and colleagues,

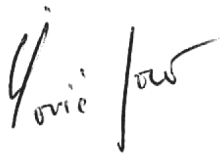
On behalf of Balkan Clinical Laboratory Federation it is my great pleasure to invite you to take part on this great scientific event to be held on 27-31 October 2019 in Antalya - Turkey.

Gear up for an exciting and informative 27th Balkan Clinical Laboratory Federation Meeting that will enable You to refresh your knowledge base, explore innovations, exchange ideas, meet other researches, friends and colleagues as well as sponsors and exibitors.

The Meeting will cover all the scientific and technological aspects of Laboratory Medicine. Ideal location to participate to very advanced scientific presentations combined with well balanced programme of oral and poster presentations, and dedicated workshops, will guarantee an efficient exchange of ideas and allow productive discussions.

These are exciting times in the world of laboratory medicine and I am sure that 27th BCLF Meeting will be a rewarding and unforgettable experience for all participants.

I look forward to meeting you all in beautiful Turkey.



Jozo Coric
BCLF President

TBS WELCOME LETTER

Dear Colleagues, Dear Friends,

On behalf of the Turkish Biochemical Society (TBS), it is a great pleasure to welcome you to Antalya and to the Joint Meeting of 27th Balkan Clinical Laboratory Federation Congress and 30th National Biochemistry Congress of TBS.

Formerly, TBS held a lot of joint international congresses with the collaboration of international organisations and BCLF. This BCLF Congress is the fifth in Turkey. The last one was in Istanbul, embedded within the Worldlab 2014.

In general, the BCLF congresses are held at the beginning of September. But we asked the colleagues from the EB of BCLF if the congress could be held at the end of October. Because we organize national biochemistry congresses every year at the end of October covering 29th October which is the anniversary of the foundation of Turkish Republic. So we celebrate this special day in our congresses every year with a greate gratefulness to Mustafa Kemal, the founder of TR. And, I am pleased that all the BCLF EB members approved our proposal. I would like to thank to all colleagues from the BCLF EB.

The scientific program of the Congress is powerful. As you know, former IFCC President **Prof. Howard Morris** would come to the Congress for the opening lecture. But it is our deep sorrow that he passed away due to a heart attack in April. We commemorate him with our deepest respect.

There are many top keynote speakers in the program such as **Khosrow Adeli (the newly elected president of IFCC, on behalf of IFCC, Canada), Mario Plebani** (opening lecturer, on behalf of IFCC, Italy), **Sverre Sandberg** (EFLM past-president, on behalf of EFLM, Norway), **Elvar Theodorsson (Sweden), Christa Cobbaert (on behalf of IFCC, Netherland), Jerca Dumic** (on behalf of FEBS, Croatia), **Hassan Bayat** (Iran), **Alexander Haliassos** (Greece), and **Tomris Ozben** (IFCC Treasurer and newly elected president of EFLM). Of course, there are a lot of foreign and Turkish outstanding scientists, difficult to name one by one, as a speaker or chair on interesting and hot topics of biochemistry / clinical biochemistry, and laboratory medicine. As total there are more than 50 speakers at the Congress.

In the scientific program, due to the high demand, we could open wide space for oral presentations. There are 116 oral presentations now. And, as usual, there are numerous (more than 200) poster presentations.

Additionally, the courses/workshops performed before or will be held during the Congress are remarkable. Two of them, the **EFLM scientific writing course** and the **miRNA Course** were successfully completed today, just before the opening. I would like to highlight the workshops during the Congress on **basic and advanced mass spec** and **Six Sigma application for analytical performance**. Another important workshop prepared by **National Metrology Institute** of Turkey on the **traceability in laboratory medicine**. Diagnostic companies also support the scientific program by their satellite symposia. Every Congress day, there are one or two industrial satellite symposia with interesting topics.

There is an important session also on 30th of October on **Nazmi Ozer Science Award**. Two young scientsits who won the awards last year will present their studies in this session. And the new award winners of this year will be announced.
Dear colleagues, geographically and culturally Turkey is a “bridge” between Europe and Asia. Therefore, and I am pleased to say that there is a greater participation to Congress than I expected with a total of >600 attendees. About 130 of them from essentially Balkan countries and other European countries such as Germany, Sweden, Norway and Croatia, but there are participants from Iran, Iraq, Syria, Pakistan, Azerbaijan etc.

The young scientists have revealed a great interest to the Congress. There are too many young scientists actively attending to the Congress with a presentation. As usual, we tried to respond to this interest by giving **registration bursary** to these young persons (We gave registration bursary to more than 60 young colleagues). We also gave young colleagues (<35 years old) an opportunity to free participation in two days EFLM scientific writing course. We are proud to be able to give this support. I hope, active participation in this Congress will prove greatly beneficial to their continuing professional development.

Dear Colleagues, historically or politically, there might be some conflicts between some of these countries in this huge region, but *science means peace or science is a means of peace* at the same time, I think. So, this meeting will contribute not only to the scientific development in lab med but also contribute to lasting and sustainable peace in this region.

I would like to highlight once again that all abstracts presented at the Congress will be published in the **Turkish Journal of Biochemistry**, the official journal of TBS and listed by SCI-E. This is also valuable for especially young colleagues, I think.

Finally, I would like to thank to all speakers, attendees, our sponsors, and the PCO, Kenes.

Dear Collegues, the Congress venue and accomodation conditions are well, the weather of Antalya is also excellent for this season. I hope all attendees will enjoy the meeting.

I wish you a successful and fruitful Congress.
Thank you for coming…
With my best regards,

Dr. Dogan Yucel
Chair of TBS and Congress

SCIENTIFIC PROGRAM

26 October 2019, Saturday

TIME	HALL B
09:00-17:00	**EFLM Course:** How to write a good scientific and professional article? ***Sverre Sandberg, Noklus, Norway - Elvar Theodorsson, Ike/Klinisk Kemi, Sweden***

27 October 2019, Sunday

TIME	HALL B
09:00-17:00	**EFLM Course:** How to write a good scientific and professional article? ***Sverre Sandberg, Noklus, Norway - Elvar Theodorsson, Ike/Klinisk Kemi, Sweden***

TIME	HALL C
09:00-17:00	**COURSE:** miRNA isolation and expression training program ***Aylin Sepici Dincel, Gazi University, Turkey - Oytun Portakal, Hacettepe University, Turkey***

TIME	HALL A
17:00-17:30	**Opening Ceremony** ***Doğan Yücel*** ***TBS President*** ***Congress President*** ***Tomris Ozben*** ***Congress Co-President*** ***BCLF Past-President and Representative of TBS*** ***EFLM President-Elect*** ***IFCC Treasurer*** ***IFCC Foundation for Emerging Nations (FEN), Board of Directors*** ***Jozo Coric*** ***BCLF President*** ***Sverre Sandberg*** ***EFLM Past-President*** ***Khosrow Adeli*** ***IFCC President-Elect***
17:30-18:30	**Opening Lecture** Quality and patient safety in laboratory medicine ***Mario Plebani, University Of Padova, Italy***

SCIENTIFIC PROGRAM

28 October 2019, Monday

Time	HALL A
08:30-10:00	**SESSION 1** ***Moderators: Diler Aslan, Turkey - Süleyman Demir, Turkey***
08:30-09:00	eApps and medical diagnostics data management ***Khosrow Adeli, University Of Toronto, Canada***
09:00-09:30	The statistical principles of laboratory data analysis ***Muhittin Serdar, Acibadem University, Turkey***
09:30-10:00	Evaluating the performance of autoverification processes using Six Sigma approach ***Abdurrahman Coskun, Acibadem University, Turkey***
10:00-10:30	**Coffee Break**
10:30-11:15	**Plenary Lecture:** ***Moderator: Tomris Ozben, Turkey*** Uncertainty in laboratory medicine ***Mario Plebani, University Of Padova, Italy***
11:15-12:00	**Industry Sponsored Symposium 1 (Beckman Coulter)** ***Moderator: Abdurrahman Coskun, Turkey*** The role of auto-verification in postanalytical process improvement ***Speaker: Ozlem Gulbahar***
12:00-13:15	**Lunch Break**
13:15-14:00	**Industry Sponsored Symposium 2 (Snibe)** ***Moderator: Mehmet Senes, Turkey*** The clinical performance of Maglumi AMH, 17-OH progesterone and B2 microglobulin ***Speaker: Pinar Eker***
14:00-14:45	**Plenary Lecture:** ***Moderator: Nazmi Ozer, Turkey*** Harmonisation in clinical laboratories and the harmonisation activities of EFLM ***Sverre Sandberg, Noklus, Norway***
14:45-16:30	**SESSION 2** ***Moderators: Erdinc Devrim, Turkey - Aylin Sepici Dincel, Turkey***
14:45-15:15	Adding value in thyroid cancer diagnostic: thyroglobulin and calcitonin measurement in fine needle aspirate washout ***Andra Caragheorgheopol, C.I.Parhon National Institute of Endocrinology, Romania***
15:15-15:45	Mass spectrometry achieving prominence in clinical medicine ***Dobrin Svinarov, Alexander Hospital, Medical University Of Sofia, Bulgaria***
15:45-16:00	SignificancAnflammatory response to obese and nonobese subjects, facts and promises ***Driton Sopa, Universitary Clinical Center Of Kosova, Kosovo***
16:15-16:25	**O-019** Evaluation of inflammatory status with procalcitonin and neopterin in healthy overweight and obese adults based on waist hip ratio ***Cigdem Sonmez, University of Health Sciences, Turkey***
16:30-17:00	**Coffee Break**
17:00-18:00	**Oral Presentations 1** ***Moderators: Cumhur Bilgi, Turkey - Oytun Portakal, Turkey***
17:00-17:15	Assessment of vitamin D status deficiency in Albanian pregnant women ***Ersida Kapllani, University Hospital Centre Mother Teresa, Albania***
17:15-17:30	Anti müllerian hormone: new roles for an established biomarker of ovarian reserve ***Demetrios Rizos, National And Kapodistrian University Of Athens, Greece***
17:30-17:45	Evidence of HbC disease in Albania. Clinical heterogeneity related to combination with other hemoglobin disorders ***Etleva Refatllari, University Hospital Center Mother Teresa, Albania***
17:45-18:00	
18:00-19:00	**APLUSTBD EXTERNAL QUALITY ASSESSMENT SCHEME** ***Moderator: Dogan Yucel, Turkey*** Why APLUSTBD EQAS? ***Dogan Yucel, Turkey*** The IFCC committee on EQA and Proficiency Testing (IFCC C-PT) Major programs and new projects. ***Alexander Haliassos, Greece*** A new approach to EQA and APLUSTBD ***Mujdat Aytekin, Turkey*** Being consistently wrong is no longer acceptable ***David Seccombe, Canada***
21:00-22:00	Yesterday, today, and tomorrow of medical laboratory services in Turkey: a discussion ***Moderator: Dogan Yucel, Turkey*** ***Ferzane Mercan, Turkey***

SCIENTIFIC PROGRAM

28 October 2019, Monday

Time	HALL B
08:30-10:00	**Oral Presentations 2** ***Moderators: Halef Okan Dogan, Turkey - Dilek Iren Emekli, Turkey***
08:30-08:40	**O-001** Thymoquinone and Sorafenib as a therapeutic combination in liver cancer: In vitro and in vivo *Eray Metin Guler, Bezmialem Vakif University, Turkey*
08:40-08:50	**O-002** Investigation of type I collagen and MMP-2 changes in mandibular bone tissue in natural development *Velid Unsal, Artuklu University Mardin, Turkey*
08:50-09:00	**O-003** Induction of APAF-1 and TRAIL by bilberry tea in HCT-116 colon cancer cell line *Burak Durmaz, Ege University, Turkey*
09:00-09:10	**O-004** Induction of apoptosis and cell cycle arrest by pomegranate extract and tangeretin in the rat mammary carcinogenesis *Huseyin Fatih Gul, Kafkas University, Turkey*
09:10-09:20	**O-005** Preparation of magnetic nanoparticle coated glutaraldehyde to reduce toxic effects of idarubicin and its effect on HL60 cell line *Hasan Ulusal, Gaziantep University, Turkey*
09:20-09:30	**O-006** The effects of overexpression of acetylcholinesterase on amyloid precursor protein and β-secretase-1 levels in Hs766T cells *Kevser Biberoglu, Hacettepe University, Turkey*
09:30-09:40	**O-007** Genome-wide CRISPR-Cas9 screening for identification of cancer essential genes in malignant pleural mesothelioma *Ece Cakiroglu, Dokuz Eylul University, Izmir, Turkey; Izmir Biomedicine and Genome Center, Izmir, Turkey*
09:40-09:50	**O-008** The importance of serum hyaluronidase measurement in discrimination of patients with prostate cancer and benign prostatic hyperplasia *Zeynep Adıyaman Koçer, University of Health Sciences, Turkey*
09:50-10:00	**O-009** Antioxidant and anti-denaturation activities of asparagus horridus grows in North Cyprus *Duygu Gencalp, Eastern Mediterranean University, North Cyprus*
10:00-10:30	**Coffee Break**
14:45-18:15	**COURSE:** Applying six sigma to analytical performance in the medical laboratories ***Hassan Bayat, Sina Medical Laboratory (Qaem Shahr), Iran***
16:30-17:00	**Coffee Break**
14:45-18:15	**COURSE:** Applying six sigma to analytical performance in the medical laboratories ***Hassan Bayat, Sina Medical Laboratory (Qaem Shahr), Iran***

SCIENTIFIC PROGRAM

28 October 2019, Monday

Time	HALL C
08:30-10:00	**Oral Presentations 3**
	Moderators: Yasemin Ucal, Turkey - Neval Aksoy, Turkey
08:30-08:40	**O-010** CA125 test request ratio in male patients
	Huriye Erbak Yilmaz, İzmir Atatürk Education and Research Hospital, Turkey
08:40-08:50	**O-011** Evaluation of tumor marker tests in a hospital setting
	Muzaffer Katar, Tokat Gaziosmanpasa University, Turkey
08:50-09:00	**O-012** Detection of preanalytical errors in blood gas analysis
	Hayat Ozkanay Yoruk, Izmir Katip Çelebi University, Turkey
09:00-09:10	**O-013** The effect of hemolysis and storage conditions on insulin stability
	Didem Barlak Keti, Erciyes University, Turkey
09:10-09:20	**O-014** Falsely low levels of unconjugated estriol: A case series of interference by anti-ALP antibodies
	Merve Sibel Gungoren, Duzen Laboratories Group, Turkey
09:20-09:30	**O-015** What if all is well except insulin? A macroinsulin case report
	Cevdet Zungun, Duzen Laboratories Group, Turkey
09:30-09:40	**O-016** Comparison of biochemical analytes in different blood collection tubes and evaluation of stability
	Fatma Demet Arslan, University of Health Sciences, Turkey
09:40-09:50	**O-017** Elevated high sensitivity troponin in the absence of coronary artery disease: a case report
	Feyza Yagmur Tekeli, Antalya Education and Research Hospital, Turkey
09:50-10:00	**O-018** Serum separation problem on gel tubes: is it a problem or a clue of some clinical conditions?
	Ahmet Ozsoy, University of Health Sciences, Turkey
10:00-10:30	**Coffee Break**
14:45-16:30	**Oral Presentations 4**
	Moderators: Guzin Aykal, Turkey - Oguzhan Zengi, Turkey
14:45-14:55	
14:55-15:05	**O-021** Simultaneous determination, quantitation and validation of the most used benzodiazepines in urine
	Cigdem Karakukcu, Kayseri City Hospital, Turkey
15:05-15:15	**O-022** Association of Nucb2/Nesfatin-1 gene polymorphism with obstructive sleep apnea severity
	Deniz Mihcioglu, SANKO University, Turkey
15:15-15:25	**O-023** The effect of lycopene on autophagy in fluoride toxicity in kidney cells
	Ayşe Usta, Van Yuzuncu Yil University, Turkey
15:25-15:35	
15:35-15:45	**O-025** The relationship between WNT signaling activity and organ attitudes in scleroderma disease sub-groups
	Ayse Kocak, Dokuz Eylul University, Turkey
15:45-15:55	**O-096** Reelin enzyme levels in emergency service suicide or self harm attempt patients
	Turgut Dolanbay, Kafkas University Health Research and Application Hospital, Turkey
15:55-16:05	**O-027** Towards the clinical implementation of pharmacogenetics in cardiology: Serbian experience
	Sanja Stankovic, Clinical Center of Serbia, Serbia; Business Academy University Novi Sad, Serbia
16:05-16:15	
16:15-16:25	
16:30-17:00	**Coffee Break**
17:00-18:00	**Oral Presentations 5**
	Moderators: Murat Cihan, Turkey - Mine Erguven, Turkey
17:00-17:10	**O-030** Inhibitory effect of glyphosate on butyrylcholinesterase and acetylcholinesterase activity
	Ayse Ulusoy, Cukurova University, Turkey
17:10-17:20	**O-031** Evaluation of Roche Accu-Chek Inform II glucose test strip system in the hospital setting
	Settar Kosova, Caycuma/Zonguldak State Hospital, Turkey
17:20-17:30	**O-032** Evaluation of urine drug screening test results between 2016-2018 years in Kanuni Education and Research Hospital Laboratory
	Nazime Cebi, University of Health Sciences, Kanuni Education and Research Hospital Laboratory, Turkey
17:30-17:40	**O-033** Pregabalin substance abuse
	Saliha Aksun, Izmir Katip Celebi University, Turkey
17:40-17:50	**O-034** The protein supplements and its inhibition of liver enzymes at athletes
	Nafija Serdarevic, University of Sarajevo, Bosnia and Herzegovina
17:50-18:00	**O-035** Effect of bariatric surgery on ghrelin-hepatosteatosis interaction: The Selcuk University Faculty of Medicine example
	Hakan Vatansev, Necmettin Erbakan University, Turkey

SCIENTIFIC PROGRAM

29 October 2019, Tuesday

Time	HALL A
08:30-10:00	**SESSION 3**
	Moderators: Ebubekir Bakan, Turkey - Berrin Bercik Inal, Turkey
08:30-09:00	Analytical performance goals
	Hassan Bayat, Sina Medical Laboratory (Qaem Shahr), Iran
09:00-09:30	Quality management: illuminating the path to ISO 15189 accreditation. A view from the Republic of North Macedonia
	Katerina Tosheska-Trajkovska, Medical Faculty/Institute Of Medical And Experimental Biochemistry, Macedonia
09:30-10:00	Quality control in research laboratory: The need for standardization
	Yasemin Ucal, Acibadem Mehmet Ali Aydinlar University, Turkey
10:00-10:30	**Coffee Break**
10:30-11:15	**Plenary Lecture:**
	Moderator: Jozo Čorić, Bosnia and Herzegovina
	Bias in clinical chemistry
	Elvar Theodorsson, Ike/Klinisk Kemi, Sweden
11:15-12:00	**Industry Sponsored Sympoisum 3 (Roche)**
	Moderator: Cem Öcal, Turkey
	Automation solutions in laboratories
	Speaker: Cigdem Karakukcu
12:00-13:15	**Lunch Break**
13:30-14:15	**Industry Sponsored Sympoisum 4 (Mindray)**
	Moderator: Muhittin Serdar, Turkey
	Clinical utility of Reticulocyte Hemoglobin and Hypochromic erythrocytes reported by Mindray BC6800 Plus hematology analyzer in the study of erythropoiesis
	Speaker: Eloísa Urrechaga
	Senior Consultant for Clinical Laboratory
14:15-15:00	**Plenary Lecture:**
	Moderator: Nada Majkic-Singh, Serbia
	Value and impact of laboratory medicine in healthcare delivery
	Khosrow Adeli, University Of Toronto, Canada
15:00-16:45	**SESSION 4**
	Moderators: Ali Unlu, Turkey - Ebru Sezer, Turkey
15:00-15:30	Lipid guidelines: emerging evidence on importance of non-fasting and postprandial lipids
	Khosrow Adeli, University Of Toronto, Canada,
15:30-16:00	Apolipoprotein profiling for addressing residual cardiovascular risk: in search of a personalized and metrologically sound answer to the latest dyslipidemia guidelines
	Christa Cobbaert, Lumc, The Netherlands
16:00-16:30	The importance of cholesterol synthesis and absorption markers determination in healthy subjects and patients with ischemic heart disease
	Tamara Gojkovic, University Of Belgrade, Serbia
16:30-16:45	Impact of redox imbalance and inflammation on activity of paraoxonase 1 and its distribution in high density lipoprotein in polycystic ovary syndrome
	Iva Perović Blagojević, KBC Dr Dragiša Mišović - Dedinje, Serbia
16:45-17:15	**Coffee Break**
17:15-18:15	**Oral Presentations 6**
	Moderators: Anyla Bulo Kasneci, Albania - Alexander Haliassos, Greece
17:15-17:30	Sensitive assessment of white blood cell functionality by novel hematological parameters
	Milena Velizarova, Medical University- Sofia, Bulgaria; Alexander University, Bulgaria
17:30-17:45	The future of cytometry in Europe
	Georgios Markopoulos, University of Ioannina, Greece
17:45-18:00	Significance of the determination of biomarkers of bone resorption and formation in patients with end stage renal disease
	Neda Milinković, University of Belgrade, Serbia
18:00-18:15	CEA monitoring in colorectal carcinoma - to the limit of the guidelines and beyond
	Yana Bocheva, Medical University- Varna, Bulgaria
20:00-23:00	**29 October, Republic Day Celebration & Networking Event**

SCIENTIFIC PROGRAM

29 October 2019, Tuesday

Time	HALL B
08:30-13:00	**COURSE:** Mass spectrometre use in clinical laboratory practice **(Basic Course)** ***Ali Unlu, Selcuk University, Turkey - Muhittin Serdar, Acibadem University, Turkey - Sedat Abusoglu, Selcuk University Faculty of Medicine, Turkey***
14:50-18:00	**Workshop:** **TUBITAK UME (National Metrology Institute of TURKEY) -** **Elements of Metrological Traceability for Laboratory Medicine**
14:50-15:10	Traceability in laboratory medicine and IVD directives ***Tomris Ozben, Akdeniz University, Turkey***
15:10-15:30	Introduction of the European Metrology Network on Traceability in Laboratory Medicine ***Muslum Akgoz, TUBITAK, Turkey***
15:30-15:50	Amino acid and organic acid CRMs for newborn screening ***Simay Gunduz, TUBITAK, Turkey***
15:50-16:10	ID-MS based reference measurement method for small analytes: vitamin D, creatinine, glucose, cholesterol, amino acids ***Mine Bilsel, TUBITAK, Turkey***
16:10-16:30	Reference methods for quantification of peptides & proteins: β-amyloid in CSF (ReMIND Project), human C-peptide, oxytocin, HbA1c, insulin, human growth hormone ***Merve Oztug, TUBITAK, Turkey***
16:45-17:15	**Coffee Break**
17:00-17:20	Latest developments on NMR; reference method for purity determination of small analytes and peptides: 17β-estradiol, folic acid, human C-peptide, oxytocin, HbA1c ***Ilker Un, TUBITAK, Turkey***
17:20-17:40	Development of a reference method for transferrin quantification in serum ***F. Gonca Coskun, TUBITAK, Turkey***
17:40-18:00	A Reference method for genetic mutation quantification of KRAS ***Muslum Akgoz, TUBITAK, Turkey***
20:00-23:00	**29 October, Republic Day Celebration & Networking Event**

SCIENTIFIC PROGRAM

29 October 2019, Tuesday

Time	HALL C
08:30-10:00	**Oral Presentations 7**
	Moderators: Banu İsbilen Basok, Turkey - Settar Kosova, Turkey
08:30-08:40	**O-036** The results in two different provinces in Black Sea Region where thalassemia screening was implemented:a rare hemoglobin variant
	Durmus Ayan, Amasya University Sabuncuoglu Serefeddin Research and Training Hospital, Amasya, Turkey
08:40-08:50	**O-037** First observation of hemoglobin Hamilton [β11(A8)Val→Ile] in Turkey
	Irem Yildiz, Cukurova University, Turkey
08:50-09:00	**O-038** Glanzmann thrombasthenia: a case report
	Aylin Haklıgor, University of Health Sciences, Turkey
09:00-09:10	**O-080** Evaluation of analytical process performance by six sigma methods in laboratories
	Dilek Iren Emekli, Erbayraktar Private Medical Laboratories, Turkey
09:10-09:20	**O-040** Determination of electrochemical behaviour of glucose-6-phosphate dehydrogenase by biosensor
	Basak Gunaşti, Cukurova University, Turkey
09:20-09:30	**O-041** Investigation of the effect of glyphosate on G6PD activity in in vitro conditions
	Kezban Kartlasmis, Cukurova University, Turkey
09:30-09:40	**O-042** The evaluation of microtubes' compatibility to automated process for complete blood count
	Ahmet Erkin Bozdemir, Health Sciences University Tepecik Training and Research Hospital, Turkey
09:40-09:50	**O-043** Design of a new biosensor for the determination of ferric iron in blood
	Ahmet Ilhan, University of Cukurova, Turkey
09:50-10:00	**O-044** Correlation Between LUC % and Thyroid Function Tests
	Arzu Kosem, Ankara City Hospital, Turkey
10:00-10:30	**Coffee Break**
14:45-16:30	**Oral Presentations 8**
	Moderators: Sevil Kurban, Turkey - Emre Avci, Turkey
14:45-14:55	**O-045** The effect of Rhamnetine against to ischemia-reperfusion injury in the kidney
	Mustafa Nisari, University of Nuh Naci Yazgan, Turkey
14:55-15:05	**O-046** The protective effect of resveratrol against cyclosporine A-induced oxidative stress and hepatotoxicity
	Ilknur Bingul, Istanbul University, Turkey
15:05-15:15	**O-047** Thiol/Disulphide balance and Ischemia-modified albumin levels in female with iron deficiency anemia
	Emre Avci, Hitit University, Turkey
15:15-15:25	**O-048** Effect of hibernation on oxidative equilibrium in ground squirrels
	Tulay Pekmez, Hitit University, Turkey
15:25-15:35	**O-049** Cellular protection by Phlomis Species in H2O2-induced oxidative Stress
	Derviş Birim, Ege University, Turkey
15:35-15:45	**O-050** Neuroprotection by optimized system extracts of Morus nigra L. Fruits in L-DOPA-induced toxicity
	Gizem Kaftan, Ege University, Turkey
15:45-15:55	**O-051** Dynamic thiol-disulphide balance and thioredoxin reductase enyzme levels in patients with chronic kidney disease
	Huseyin Erdal, Hatay Mustafa Kemal University, Turkey
15:55-16:05	**O-052** Protective role of lycopene in experimental heart ischemia reperfusion model
	Busra Citil, Sutcu Imam University, Turkey
16:05-16:15	**O-053**
16:15-16:25	**O-054**
16:45-17:15	**Coffee Break**
17:00-18:00	**Oral Presentations 9**
	Moderators: Bahadir Ozturk, Turkey - Aylin Hakligor, Turkey
17:00-17:10	**O-055** Oxidative status in degenerated painful ıntervertebral disc samples: variability with respect to duration of symptoms and type of disease
	Hatice Kopar, Sutcu Imam University, Turkey
17:10-17:20	**O-056** The effect of turmeric on GPER1 and oxidative/nitrosative stress biomarkers in cardiac ischemia reperfusion
	Seda Ikikardeş, Sutcu Imam University, Turkey
17:20-17:30	**O-057** The impact of acupuncture treatment on dynamic thiol–disulphide homeostasis and ischemia-modified albumin levels to assess
	Yasemin Gunduztepe, Gazi University, Turkey
17:30-17:40	**O-058** Effect of N-acetylcysteine on cisplatin induced apoptosis in rat kidney
	Seyda Seydel, Nigde Omer University, Turkey
17:40-17:50	
17:50-18:00	**O-060** Thiol-disulfide homeostasis in diabetic microvascular complications
	Cuma Mertoglu, Erzincan University, Turkey
20:00-23:00	**29 October, Republic Day Celebration & Networking Event**

SCIENTIFIC PROGRAM

30 October 2019, Wednesday

Time	HALL A
08:30-10:00	**SESSION 5**
	Moderators: Z. Gunnur Dikmen, Turkey - Sabahattin Muhtaroglu, Turkey
08:30-09:00	IFCC, C-RIDL; The current concept and future plans for reference intervals and decision limits
	Yesim Ozarda, Uludag University, Turkey
09:00-09:30	Developing a roadmap for laboratory test utilization management program
	Sedef Yenice, G Florence Nightingale Hospital, Turkey
09:30-10:00	Threat of chemical weapons in Syria conflict and its impact on Balkan region along with the health and laboratory management system
	Levent Kenar, University Of Health Sciences, Turkey
10:00-10:30	**Coffee Break**
10:30-11:15	**Plenary lecture:**
	Moderator: Muslum Akgoz, Turkey
	The new IVD regulation 2017/746 and consequences for laboratory medicine
	Christa Cobbaert, Lumc, The Netherlands
11:15-12:00	**Industry Sponsored Symposium V (Archem)**
	Moderator: Mujdat Aytekin, Turkey
	HbA1c immunoturbidimetric test: CRM concept, standardization and interference studies
	Speaker: Mehmet Salih Uca
12:00-13:15	**Lunch Break**
13:15-14:00	**Oral Presentations 10**
	Moderators: Ali Unlu, Turkey - İlhan Yaylım, Turkey
13:15-13:25	**O-061** Biological variation in clinical practice: bridge between laboratorians and clinicians
	Fatma Hande Karpuzoglu, Acibadem Labmed, Turkey
13:25-13:35	**O-062** Using the model of quality indicators: a pilot study
	Oguzhan Zengi, Bagcilar Research and Training Hospital, Turkey
13:35-13:45	**O-063** National guidelines for the preparation, distribution and testing of purified water for clinical laboratories
	Oytun Portakal, Hacettepe University, Turkey
13:45-13:55	**O-064** Evaluation of CKD-EPI Pakistan equation for estimated glomerular filtration rate (eGFR) in Pakistan
	Sibtain Ahmed, The Aga Khan University, Karachi Pakistan
14:00-14:45	**Plenary Lecture:**
	Moderator: Ferhan Sagin, Turkey
	Galectin-3: from molecule to biomarker and back
	Jerka Dumic, University of Zagreb Faculty of Pharmacy and Biochemistry, Croatia
	'The FEBS National Lecturer'
14:45-16:30	**SESSION 6**
	Moderators: Fatma Taneli, Turkey - Sedat Abusoglu, Turkey
14:45-15:15	Serum non-coding RNA profiling as a promising diagnostic approach
	Christos Tsatsanis, University Of Crete Medical School, Greece
15:15 - 15:45	Ethical challanges in (pharmaco) genetics
	Marija Hiljadnikova-Bajro, Ss Cyril And Methodius University, Macedonia
15:45 - 16:00	The relationship between adiposity parameters and hsC-reactive protein values in overweight and obese women
	Aleksandra Atanasova Boshku, University Clinic For Gynecology And Obstetrics, Macedonia
16:00-16:10	**O-113** Transcriptomic meta-analysis in pancreatic ductal adenocarcinoma reveals therapeutic targets and diagnostic biomarkers
	Sevcan Atay, Ege University, Turkey
16:30-17:00	**Coffee Break**
17:00-18:00	**Oral Presentations 11**
	Moderators: Ferhan Sagin, Turkey - Dogan Yucel, Turkey
	Yasemin Aksoy, Turkey - Ozlem Dalmizrak, Turkey
17:00-17:15	**2018 Nazmi Ozer Award Recipient Presentation**
	Molecular demultiplexer as a terminator automaton
	Gurcan Gunaydın, Turkey
17:15-17:30	**2018 Nazmi Ozer Award Recipient Presentation**
	Photodynamic activity properties of novel BODIPY compound against colorectal cancer cell line
	Burak Barut, Karadeniz Technical University, Turkey
17:30-17:45	**2019 Nazmi Ozer Awards**

SCIENTIFIC PROGRAM

30 October 2019, Wednesday

Time	HALL B
08:30-13:00	**COURSE:** Mass spectrometre use in clinical laboratory practice **(Advance Course)** ***Ali Unlu, Selcuk University, Turkey - Muhittin Serdar, Acibadem University, Turkey - Sedat Abusoglu, Selcuk University Faculty of Medicine, Turkey***
13:15-14:00	**Oral Presentations 12** ***Moderators: Fatma Demet Arslan, Turkey - Muammer Yucel, Turkey***
13:15-13:25	**O-065** The local technical validation of Barricor™ tube that uses a mechanical separator ***Kamil Taha Ucar, Istanbul Gaziosmanpasa Taksim Training and Research Hospital, Turkey***
13:25-13:35	**O-066** The utility of preanalytical quality indicators: a Turkish survey study ***Hikmet Can Cubukcu, Erzurum Maresal Cakmak Devlet Hastanesi, Turkey***
13:35-13:45	**O-067** A web-based application for management of quality control data ***Deniz Ilhan Topcu, Başkent University, Turkey***
13:45-13:55	**O-068** Quality control application for CBC parameters by 'Average of Normals' method ***Ilknur Alkan Kusabbi, Health Sciences University, Turkey***
14:45-16:35	**Oral Presentations 13** ***Moderator: Meral Yuksel, Turkey***
14:45-14:55	**O-069** Evaluation of the most common rejection reasons in the preanalytical process at our laboratory using six sigma analysis ***Mehmet Akif Bozdayi, Gaziantep University, Turkey***
14:55-15:05	**O-070**
15:05-15:15	**O-071** Analytical performance of Cobas 6500 for predicting urinary tract infection ***Esra Firat Oguz, Ankara City Hospital, Turkey***
15:15-15:25	**O-072** A comparison of Sysmex UF-5000 flow cytometer and Fuchs-Rosenthal Chamber in urine sediment analysis ***Ozlem Unay Demirel, Bahcesehir University, Turkey***
15:25-15:35	**O-073** Determination of serum carbamazepine by tandem mass spectrometry ***Duygu Eryavuz Onmaz, Selcuk University, Turkey***
15:35-15:45	**O-074** 3D placental barrier models: a novel cryogel based method ***Aysun Kilic Suloglu, Hacettepe University, Turkey***
15:45-15:55	**O-075** Antioxidant effects of flavonoid neoeriocitrin on streptozotocin-induced INS-1E cell diabetic model ***Elif Karacaoglu, Hacettepe University, Turkey***
15:55-16:05	**O-076** In vitro investigation of Argiope bruennichi derived spider silk materials ***Secil Karahisar Turan, Hacettepe University, Turkey***
16:05-16:15	**O-077** MicroRNAs in patients with type 2 diabetic nephropathy ***Kadriye Akpinar, Pamukkale University, Turkey***
16:15-16:25	**O-078** Differentiation of Osteopetrotic IPSC to Osteoclasts:Comparision of Osteopetrotic&Healthy Osteoclast ***Inci Cevher, Hacettepe University, Turkey***
16:25-16:35	**O-079** Monodisperse-porous metal oxide microspheres with peroxidase/oxidase mimetic activity as a new tool for biomolecule determination ***Sevim Eda Ogut, Hacettepe University, Turkey***
16:30-17:00	**Coffee Break**
18:00-19:00	**Oral Presentations 14** ***Moderators: Oguzhan Zengi , Turkey - Deniz Ilhan Topcu, Turkey***
18:10-18:20	**O-081** Evaluation of analytical quality of cardiac biomarkers in the emergency laboratory by sigma metrics ***Saadet Kader, Karapinar State Hospital, Turkey***
18:20-18:30	**O-083** Automated vitamin D immunoassay comparison with LC-MS/MS method ***Ercan Saruhan, Mugla Sitki Kocman University, Turkey***
18:30-18:40	**O-084** Calculation of measurement uncertainty of three different biochemistry parameters ***Seren Orhan, Gaziantep University, Turkey***
18:40-18:50	**O-085** Development of a LC/MSMS method for quantification of adrenal-derived 11-oxygenated 19-carbon steroids ***Ali Yaman, Marmara University, Turkey***

SCIENTIFIC PROGRAM

30 October 2019, Wednesday

Time	HALL C
08:30-10:00	**Oral Presentations 15**
	Moderators: Ercan Saruhan, Turkey - Bagnu Orhan, Turkey
08:30-08:40	**O-086** Structural bioinformatics approach in bioactive peptide research: tomato vicilin case study
	Burcu Kaplan Turkoz, Ege University, Turkey
08:40-08:50	**O-087** In silico prediction of antidepressant-binding sites on human glutathione reductase
	Kerem Terali, Near East University, Cyprus
08:50-09:00	**O-088** Smart approval service for biochemical tests
	Ali Ozen Akyurek, Ventura Software Inc., Ankara, Turkey
09:00-09:10	**O-089** Evaluation of saliva kallikrein-8 levels related with stress
	Rabia Semsi, Gazi University, Turkey
09:10-09:20	**O-090** The evaluation of ADAMTS-1 and ADAMTS-13 levels at coronary collateral circulation
	Abdulhakim Hasan Gul, Onsekiz Mart University, Turkey
09:20-09:30	**O-091** Apelin and other adipokines as potential biomarkers in myocardial ischemia
	Mehmet Ali Gul, Ataturk University, Turkey
09:30-09:40	**O-092** Relationship between platelet activating factor acetylhydrolase and cardiac valvular calcification in dialysis patients
	Serkan Bolat, University of Health Sciences, Turkey
09:40-09:50	**O-093** Determination of ADMA and ghrelin levels as a marker of endothelial dysfunction in asthma patients
	Burcu Baba, Yüksek İhtisas University, Turkey
09:50-10:00	**O-094** Antimicrobial and antioxidant activities of Lactarius deliciosus
	Elif Sevinc, Hitit University, Turkey
10:00-10:30	**Coffee Break**
13:15-14:00	**Oral Presentations 16**
	Moderators: Aysegul Cort, Turkey - Aysun Kilic Suloglu, Turkey
13:15-13:25	**O-095** Telmisartan and irbesartan alleviate methylglyoxal-induced elevation of MG-H1 in VSMCs
	Mustafa Kirca, Kutahya Health Sciences University, Turkey
13:25-13:35	**O-116** Drug-induced (quinine) acute hepatitis with high level of serum vitamin B12
	Ozlem Ozun, University of Health Sciences Suat Seren Chest Diseases and Surgery Training and Research Hospital, Turkey
13:35-13:45	**O-097** Relationship between lipoprotein(a) and other lipids in children
	Fatime Merdan, Child Health and Diseases Training and Research Hospital, Turkey
13:45-13:55	**O-098** Relationship between B-HCG and LUC% levels
	Funda Eren, Ankara City Hospital, Turkey
14:45-16:35	**Oral Presentations 17**
	Moderators: Cigdem Sonmez, Turkey - Burak Barut, Turkey
14:45-14:55	**O-099** Biological variation of beta-trace protein, a novel marker for eGFR along with traditional markers
	Banu Isbilen Basok, Health Sciences University, Turkey
14:55-15:05	**O-100** Calculation of APTTandPT reference intervals from patient data and evaluation of preoperative test utilisation in surgicalpatients
	Neslihan Cihan, Ankara Health Research and Training Hospital, Turkey
15:05-15:15	**O-101** ICD code specific normal ranges are needed, particularly in total bilirubin in this case
	Ozgur Aydin, Kepez Public Hospital, Turkey
15:15-15:25	**O-102** Pending laboratory tests at discharge in emergency department
	Murat Alisik, Polatli State Hospital, Turkey
15:25-15:35	**O-103** The effect of blood lactate levels on mortality in patients with sepsis
	Kamile Yucel, KTO Karatay University School of Health Sciences, Turkey
15:35-15:45	**O-104** The anti-inflammatory effects of orexin receptor antagonist on endotoxemia induced sepsis model
	Evren Kilinc, Acibadem Mehmet Ali Aydinlar University, Turkey
15:45-15:55	**O-105** Correlation of CRP with blood-based inflammatory markers; large cohort study
	Sibel Soylemez, Gazi University, Turkey
15:55-16:05	**O-106** Serum cytokine and complement levels in ALS and their association with LRP antibody positivity
	Murat Giris, Istanbul University, Turkey
16:05-16:15	**O-107** The relationship between standard sedo-analgesia implementation and serum procalcitonin levels in intensive care unit
	Yesim Guvenc Demiragci, Manisa Celal Bayar University, Turkey
16:15-16:25	**O-108** Midkine can not be accepted as a new biomarker for the diagnosis and the treatment of unexplained female infertility
	Mine Erguven, İstanbul Aydın University, Turkey
16:25-16:35	**O-109** Perspective of C-peptide from diabetes window
	Saadet Kader, Selcuk University, Turkey
16:30-17:00	**Coffee Break**
18:00-19:00	**Oral Presentations 18**
	Moderators: Berrin Bercik Inal, Turkey - Feyza Yagmur Tekeli, Turkey
18:00-18:10	**O-110** *Development and validation of a biosensor for measurement of serum hypoxia-inducible factor-1*
	Zihni Onur Uygun, Ege University, Turkey
18:10-18:20	**O-111** A fast and convenient UPLC - MSMS Method for routine analysis of GALT activity from dried blood spot
	Muhammet Topbaş, Ege University, Turkey
18:20-18:30	**O-112** Magnetic bead based electrochemical food and enzyme activity analysis by using SPE dependent immunosensors
	Ebru Saatci, Erciyes University, Turkey
18:30-18:40	
18:40-18:50	**O-114** Assessment of vitamin D levels in Şanlıurfa region
	Oruc Aslan, Harran University Medical School, Turkey
18:50-19:00	**O-115** The role of HDL-associated MPO and PON-1 for coronary artery disease in Hashimoto thyroiditis
	Gizem Uncu, Hitit University, Turkey

SCIENTIFIC PROGRAM

31 October 2019, Thursday

Time	HALL A
09:00-10:30	**SESSION 7**
	Moderators: Gultekin Yucel, Turkey - Güzin Aykal, Turkey
09:00-09:30	Clinical and laboratory approach to inborn errors of metabolism
	Ali Dursun, Hacettepe University, Turkey
09:30-10:00	Metabolomics and biomarkers in inborn errors of metabolism
	Incilay Lay, Hacettepe University, Turkey
10:00-10:30	Genetic technologies in inborn errors of metabolism
	Didem Yucel Yılmaz, Hacettepe University, Turkey
10:30-11:00	**Coffee Break**
11:00-11:30	**CLOSING CEREMONY OF THE CONGRESS**

INVITED SPEAKERS ABSTRACTS

Quality and patient safety in laboratory medicine

Mario Plebani
University of Padova-Department of Laboratory Medicine, Italy

The path leading to quality and patient safety in laboratory medicine is infinite, since it must be ensured that each and every step in the total testing process (TTP) is correctly performed, thus guaranteeing a valuable medical decision making process and effective patient care. Laboratory-associated error has a completely different meaning today than it did a century ago. At that time the term referred to defects in the analytical performance of the test itself, the so-called analytic phase. The new millennium has hailed a formidable improvement in the analytical phase with a 100-fold reduction in error rates, thanks to an improved standardization of analytic techniques and reagents, advances in instrumentation and information technologies, as well as to the availability of more qualified and better trained staff. In addition, this achievement is due to the development and introduction of reliable quality indicators (QIs) and quality specifications for the effective management of analytical procedures by adopting internal quality control programs and attending external quality assurance (EQA/PT) schemes. According to recent evidence, most errors fall outside the analytical phase, in fact, the extra-analytical steps (both pre- and post-analytical) have been found to be more vulnerable to the risk of error. It needs, therefore, to evaluate all the steps in TTP, whether or not they fall under the direct control of laboratory personnel, with the ultimate goal being to improve, first and foremost, quality and safety for patients. Quality indicators (QIs) are fundamental tools for enabling users to quantify the quality of all operational processes by comparing it against a defined criterion. According to the International Standard for medical laboratories accreditation, the laboratory shall establish and periodically review QIs to monitor and evaluate performance throughout critical aspects of pre-, intra-, and post-analytical processes. A consensual agreement on a possible list of QIs has been recently achieved after revising the model of quality indicators (MQI) developed by the Working Group on "Laboratory Errors and Patient Safety" of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in two Consensus Conferences organized in 2013 and 2016. The consensually accepted list of QIs, which takes into consideration both their importance and applicability, should be tested by all potentially interested clinical laboratories to identify further steps in the harmonization project. The data collected in the last few years, have already allowed us to establish tentative performance specification for extra-analytical phases and to demonstrate that error rates may decrease after QIs monitoring and performing appropriate corrective actions.

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Electronic apps and medical diagnostics data management

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Laboratory medicine is a domain which offers a unique opportunity to analyze objective patient laboratory data and enable ready communication to both healthcare workers as well as patients. In recent years, an increasing number of web-based and mobile applications has been developed to improve access to laboratory test information and test result interpretation. They range from simple apps that provide reference lab value information to complex medical diagnostics data management. As examples, the "eLab" developed by Tru-Solutions Inc. is a comprehensive medical diagnostic center and lab management software that provides a user friendly interface and access control. It is linked iMedDx.com to allow flexible patient search and selection and includes an eLab Dashboard on mobile/tablet, allowing patients and labs/hospitals access to lab reports online. The Davis's Laboratory & Diagnostic Tests medical app provides another useful app with a wide-breadth of tests, as well as guidance on how to counsel and collect tests. The app is available on multiple platforms including the iPhone/iPad, Android and Blackberry. The "LabGear" is a medical lab reference app providing a pocket tool for medical laboratory test and is integrated with MedCalc with normal lab value reference information for over 200+ lab tests. There are several other medical apps that provide reference lab values including CALIPER, MedRef, Normal Lab Values, and Lab Tests. The CALIPER App has been developed in our laboratory for paediatricians, family physicians, and other healthcare workers worldwide. It is a user friendly and easy tool to assess a child's laboratory test results using the latest reference value database developed based on a study of thousands of healthy children and adolescents. The CALIPER apps allow pediatricians & family physicians to interpret laboratory test results for over 170 medical laboratory tests in children and adolescents using a comprehensive database of pediatric data

The statistical principles of laboratory data analysis

Muhittin A. Serdar, Prof. Dr.
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The majority of scientists (app 70%) are often afraid of statistics. Because most scientists do not fully understand statistics, they tend to either overestimate or underestimate. Unless concepts of statistics are thoroughly understood and comprehended, critical evaluation of scientific research will hardly be adequate and efficient.
Statistics, as defined by the American Statistical Association, is "the science of learning from data, and of measuring, controlling and communicating uncertainty". Briefly, statistics is the science concerned with developing and studying methods for collecting, analyzing, interpreting, and presenting empirical data. In this lecture, we will briefly discuss the statistics from the laboratory specialist's point of view.
Descriptive (table, figure, etc.) and inferential statistics (group comparison, correlation, regression, etc.) are two broad categories in the field of statistics. A third group, especially useful for Laboratory Specialists is "specific statistics", which consists of methods for validation, verification, reference intervals, biological variation, quality control statistics, etc.
One of the difficulties in understanding statistics is the "p-value". A lower p-value is generally interpreted as a stronger relationship or differences between two and all variables. Nevertheless, statistical significance means that, it is unlikely that the null hypothesis is correct. To understand the strength of the difference between two groups (control vs. experimental) a researcher needs to calculate the effect size.
The concept of "effect size" enables the readers to understand the magnitude of differences found; however, statistical significance examines the probability of an outcome to occur by chance alone.
The words "data", "information" and "knowledge" are sometimes used interchangeably. It is essential to understand how "knowledge" differs from "data" and "information", and to understand what "knowledge management" can add to clinical practice.
Data Science refers to the umbrella of techniques by which, one is trying to extract information and insights from data.
Data mining (DM) is the process of analyzing unknown patterns of data according

to different points of view for categorization into valuable information, which is collected in common areas, for example, data warehouses, for efficient analysis, data mining algorithms, facilitation decision making, and other information requirements to cut costs and increase revenue ultimately. DM is also known as data discovery and knowledge discovery.
DM and Big Data (BD) are two different concepts. BD is a term, which refers to a large amount of data whereas DM refers to deep drive into the data to extract the critical knowledge from a small or large amount of data.
Data Mining and Big Data are essential for clinical laboratories. A medium-sized laboratory can generate 3 to 4 million patient test results a year and also, each one of those results has related data that never make it to the chart. Our goal in analyzing these laboratory and clinical data is to see whether we can uncover ways to improve not only laboratory practice, but clinical practice all together. That is, in addition to ensuring the accuracy, precision, and turnaround times of laboratory results.
There are lots of softwares for laboratory statistics; some of which we will be investigating during this course. These are *Ep Evaluator*, *Analyse-It*, *MedCalc*, *XLSTAT*, *QI Macros*, *Minitab*, *SPSS*, *Stata*, *SAS*, *R*. All of them, except for "*R*", are usually expensive commercial softwares. When analyzed in terms of laboratory statistics, it is observed that *Analyze It*, *Ep Eva*luator and sometimes *MedCalc* are more convenient than others. While it may not be the ideal software for advanced statistics and Data Mining studies; *Analyze It* appears to be the most user-friendly software for basic laboratory statistics. Being an open-source and free software, that enables considerable flexibility; "R" definitely stands out as an advantageous software among the others. However, it is not as user-friendly, and certainly requires significant experience.
As a result;
· The problem of fear of statistics should be overcome.
· It is important not to confuse statistical significance with clinical significance.
· A clear understanding of various information technology tools (computer, software, LIMS, HIMS) will enable appropriate and efficient analysis.
· Each specialist must be able to make and evaluate basic statistics and laboratory statistics. Laboratory scientists should learn to apply statistical tools correctly, interpret the findings correctly and get an idea about the possibilities of analyzing research questions using statistics.
· A single software cannot solve all our problems. Sufficient comprehension of and substantial experience in Microsoft Excel and SPSS (or other general software) is a must. Where possible, data mining should also be carried out.
· Big data and data mining are critically important to us. However, it is important to note that analysis of the Big Data alone will not guarantee better outcomes. Overwhelming the clinicians with unrestrained volumes of data bears the risk of complicating the separation of signal from the noise.

Evaluation the Performance of Autoverification Processes Using Six Sigma Approach

Abdurrahman Coskun
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One of the main objectives of the quality is to minimize the error rates to a negligible level. The literature of laboratory errors goes back to 1950s (1,2). In 1999 the report of Institute of Medicine (US) 'To Err is Human: Building a Safer Health System' broke the silence on medical errors, and created awareness in the public and healthcare sector. Later in 1915 we learned that the big picture was worse and medical error is the third leading cause of death in US (3). In total testing process (TTP), the error rates are higher in the phases where human interventions are higher such as pre-pre and post-post analytical phases. To decrease error rates of each phases of TTP, we should decrease human interventions and implement laboratory automation and artificial intelligence.
Autoverification (AV) of test results decrease error rate and increase the efficiency of laboratory and patients' safety. In addition to verification test results, a well-designed AV system use patient-related clinical information, instrumental messages and flags to help physicians to interpret test results correctly and consequently decrease the error rate in post-post analytical phase.
Six Sigma methodology has been evolved from total quality management and created a revolution in quality management in new millennium. It is not only a statistical tool but also provide problem solving methods by using the approach of define, measure, analyze, improve and control (DMAIC) cycle. In each phase of this approach statistical procedures are used to evaluate the performance of the phase and help us to take the corrective actions. In addition to DMAIC, the performance of a process can be measured objectively using sigma metric (SM). SM denotes the number of standard deviations (SD) of the process fit between the target and upper/lower tolerance limits. 6 sigma represents the world class quality and in this process only 3.4 errors or defects occur per million opportunities (DPMO). SM can be converted to DPMO and inversely DPMO can be converted to SM. This flexibility enabled the application of the Six Sigma Methodology to a broad area such as industry, business, healthcare sector etc..
It has been shown that applying the principles of Six Sigma methodology (DMAIC) to AV systems improved turn-around time and reduced time for manual verification (4). For the ideal AV system, DMAIC principles should be taken into consideration while developing a suitable system compatible with the realities of the laboratory, and an objective criteria such as SM should be used to measure the performance of the system. A detailed data analysis using fishbone diagram and Pareto chart can be used to evaluate the performance of AV systems.
In conclusion, AV systems designed on the principles of Six Sigma methodology will increase the performance of laboratory, decrease error rate and contribute patients' safety significantly. Additionally, the performance of AV systems should be measured using an objective criteria such as SM.

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Uncertainty in laboratory medicine

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Medical laboratories should guarantee that their measurement procedures (MPs) results are fit for clinical purposes, and this requirement calls for the long-term monitoring of the quality and reliability of results. Since its inception, the International Standard ISO 15189 for medical laboratory accreditation has called for the calculation of measurement uncertainty (MU) to be included in each MP. Interestingly, because MPs are used to describe the whole measurement process, including the specific analytical procedure, all processes which contribute to uncertainty in the test results should be considered when calculating MU. The international vocabulary of metrology (VIM) has defined MU as a "nonnegative quantity that characterizes the dispersion of the values that could reasonably be attributed to the measurand". For a given test result, MU thus represents the interval associated with a defined probability in which the true result should lie. In addition, this interval should fall within limits which guarantee fitness for the clinical purpose of the tests in question. Measurement uncertainty goals for defining fitness-for-purpose limits may be based on clinical outcome studies, biological variation, state of the art, recommendations from an expert group or professional opinions. The components which require consideration in calculating MU are systematic error (bias) and random errors. Bias is inversely related to the degree of trueness of a measurement, while random error represents imprecision and is defined as the standard deviation of a series of measurements. I would like to provide some usable practical procedures regarding the MU estimation for a series of MPs, routinely used in medical laboratories. In particular, for imprecision component its estimation appears to be a reliable estimation of MU if the correct interpretation of the lab test result is guaranteed on the basis of its clinical purpose. For the bias component, the development of a practical solution for including bias in MU estimation allowed us to derive a standardized approach that considers the source of the bias reference and whether and how bias can be calculated.
In addition, MU is an important information to improve the appropriate interpretation of laboratory results and reduce the risk of errors. In fact, diagnostic uncertainty may derive from incomplete information in laboratory reports, leading to an increased risk of inappropriate interpretation of laboratory data.
Therefore, MU has two intended uses: for laboratory professionals, it gives information about the quality of measurements, providing evidence of the compliance

with analytical performance characteristics; for physicians (and patients) it may help in interpretation of measurement results, especially when values are compared with reference intervals or clinical decision limits, providing objective information.

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Harmonisation in clinical laboratories and the harmonisation activities of EFLM

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Harmonisation is likely to be an important contributor to ensure high quality laboratory testing, thus potentially improving patient outcome. Efforts for harmonisation must be made in the total testing process, from test requesting to communication of the laboratory test results and its consequences to the patient. In this article, suggestions are given about what level of harmonisation is possible at the various steps of the testing process, who could be responsible for facilitating and monitoring the effects of harmonisation, and what are likely barriers to achieving harmonisation. Harmonisation can be achieved at local, national and international levels, and will be most challenging when it involves more than one profession as in the extra-analytical phases. Key facilitators will be laboratory associations, regulatory bodies and accreditation systems, whereas barriers are likely to be reimbursement systems or economic factors, opinion leaders and manufacturers. A challenge is to try to turn barriers into facilitators. Harmonisation effects can in most settings be monitored by external quality assurance organisations provided that schemes are expanded to cover all relevant steps and phases. We must combine our efforts, both within our profession as well as in cooperation with others, to achieve harmonisation of the total testing process, in the best interests of the patient. The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) has initiated many harmonization activities in all phases of the examination process. The EFLM is dealing with both the scien- tific and the educational aspects of harmonization, with the intention of disseminating best practice in laboratory medicine throughout Europe. Priorities have been given (1) to establish a standard for conducting and assessing biological variation studies and to construct an evidence based EFLM webpage on biological variation data, (2) to harmonize preanalytical procedures by producing European guidelines, (3) to improve test ordering and interpretation, (4) to produce other common European guidelines for laboratory medicine and play an active part in development of clinical guidelines, (5) to establish a common basis for communicating laboratory results to patients, (6) to harmonize units of measurement throughout Europe, (7) to harmonize preanalytical procedures in molecular diagnostics and (8) to harmonize and optimize test evaluation procedures. The EFLM has launched a new database for biological variation study (www.eflm.eu) and also the 5th version of the European Syllabus to help the education of European Specialists in Laboratory Medicine (EuSpLM).

Adding value in thyroid cancer diagnostic: thyroglobulin and calcitonin measurement in fine needle aspirate washout

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The diagnostic approach to thyroid cancer (TC) is one of the most challenging issues in the oncology of the endocrine system because of its growing incidence, the difficulty in distinguishing benign from malignant non-functional thyroid nodules and in accurately establishing cervical lymph node involvement during preoperative staging, as well as identifying later recurrences.

Neoplastic transformation can occur in either the follicular cells of the thyroid, generating differentiated tumors (papillary thyroid carcinomas (PTCs), follicular thyroid carcinomas (FTCs), Hürthle cell carcinomas), or rarely, poorly differentiated and anaplastic thyroid carcinomas, or in the parafollicular cells of the gland, producing medullary thyroid carcinomas (MTCs).

Accurate selection for surgery of thyroid nodules at risk for malignancy, as well as avoiding the adverse effects of overdiagnosis and overtreatment it is of critical clinical importance. Optimal diagnosis and stratification of TCs needs less-invasive, specific, reliable and clinically relevant biomarkers.

Fine-needle aspiration biopsy (FNAB) of thyroid nodules or lymph nodes is a useful and safe tool and it is considered the gold-standard method in TC diagnosis and monitoring. Despite its high accuracy, in 10-30% of the cases the cytology is inconclusive. Measurement of biochemical tumor markers (thyroglobulin (Tg), calcitonin (Ct), recently CYFRA 21-1) in washout fluids from FNAB of lymph nodes or thyroid nodules is recommended as an ancillary tool for the management of TC patients.

In differentiated thyroid cancer (DTC) Tg measurement in FNAB washout (FNAB-Tg) of a suspect lymph node may increase the diagnostic sensitivity and specificity particularly in those cases in which the lymph nodes are cystic, cytological evaluation of the lymph node is indeterminate, or the cytological and sonographic evaluations are divergent (i.e., normal cytological biopsy of a large lymph node with microcalcifications).The diagnostic performance of Tg-FNAB compares favorably with cytology, having superior results in athyreotic patients.

For the diagnosis of MTC the recommendations of the American Thyroid Association (2016) is that FNAB inconclusive results should be followed-up by Calcitonin (Ct) measurement in the FNAB washout fluid (FNAB-Ct), in addition to IHC staining of the FNAB sample for several tumor markers (Ct, chromogranin, CEA).

However, the lack of standardization of FNAB-biomarkers measurements (patient selection, technique of sampling, standardization of the analytical methods - e.g. washout matrix, samples processing and storage, assays, antibodies and/or biotin interferences, cut-off values) rises potential difficulties in interpreting data and have an important impact on clinical decision.

We evaluated the analytical performance of FNAB-Tg and FNAB-Ct immunoassays in our laboratory. For both determinations the washout was performed by rinsing the needle with 1 ml saline solution 0.9% immediately after the biopsy's cellular component was expelled for the cytological examination. No matrix interference was demonstrated with saline solution either for Tg, or for Ct (LOB = 0.04 ng/ml/<0.5 pg/ml, LOD = 0.046 ng/ml/0.55 pg/ml, respectively), when measured with an immunoelectrochemiluminiscent method. Validation parameters (accuracy, precision, reproducibility, recovery, dilution linearity) fulfilled the acceptance criteria.

Besides analytical validation, studies for the clinical validation are ongoing, in the attempt to identify the best cut-offs for FNAB-Tg and FNAB-Ct.

Our experience so far confirms that a reflex strategy would be most cost-effective: negative or non-diagnostic or indeterminate cytology cases should be reflexed to FNAB-Tg/Ct, while positive cytology cases do not need measurement of tumor markers in FNAB.

The management of TC patient may be improved by a genomic approach, various diagnostic and prognostic molecular markers (BRAF, PAX8/PPRG, RAS, TP53, TERT promoter, mutations and RET/PTC rearrangements) being available; the benefit of the extent of their analysis in FNAB samples should be further evaluated.

A generally accepted standardization of tumor markers measurement in FNAB is required and the results should be integrated in the context of the full clinical, imagistic and histological picture.

Key words: Thyroid cancer, FNAB, thyroglobulin, calcitonin

Mass spectrometry achieving prominence in clinical medicine

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There is an extraordinary flood of new technologies in medicine nowadays - sophisticated diagnostics based on mass spectrometry, genome assays and cell sorting platforms are driving the technological transfer and promote the entrance of individualized patient management in clinical practice. Mass spectrometry (MS) could be viewed as one of the major tools that achieve prominence in clinical medicine. GC-MS was the starting of MS for biochemical research and clinical analysis, and still remains a working horse for clinical toxicology. LC-MS/MS (QQQ) is the today's most utilized analytical platform, but high-resolution MS systems are also employed to resolve challenging analytical demands. MALDI-TOF platforms are routine instruments in medical microbiology laboratories from over 10 years now, which revolutionize diagnostics of infectious diseases, achieving ultimate speed and accuracy. Orbitrap and tandem TOF MS systems transfer proteomic and peptidomic research into clinical diagnostics with unprecedented incite and data to understand deepest pathobiochemical mechanisms of many illnesses. The great technological advance of LC-MS/MS resulted in the introduction of methods with extreme sensitivity, specificity and extended linearity range, which are simpler to use in the medical laboratories, and are based on the current reference analytical principles. Further, the ability to perform panel profiling with simultaneous measurement of bioactive compounds, their precursors and metabolites in a single sample, enormously amplifies the informative value of results, with significant improvement of patient care. Typical examples include new born screening, TDM, toxicology, endocrinology and others. There is an ultimate demand for clear differentiation of the discovery stages, selection and validation of newer biomarkers, as well as analytical method development and validation of MS techniques that are standardized to meet criteria for clinical use with post validation routine proficiency testing assessment: CLSI has issued guidance for validation and performance characteristics of LC-MS/MS methods for clinical use, which is much more stringent, compared to industrial requirements. Currently, MS is the preferred technique in central laboratories, where the expertise and the larger sample workload provide cost-effectiveness and reliability in applications. Clinical MS will flourish in the near future, with the introduction of certified commercial LC-MS assay kits, and automated analytical platforms closely resembling routine clinical chemistry analyzers. In addition, clinical MS will meet and get together chemical and anatomical pathology: MS imaging and I-knife-MS guidance in surgery, although still in research phase, open new horizons for personalized treatment and individualized patient care, with ultimate impact on precision medicine. Precision medicine (also referred to as personalized medicine), employs patient's genotype and phenotype investigation to establish individually tailored drug treatment. While genetic testing allows the physician to choose appropriate medicine, the performance of MS assays provides the patient's actual phenotype, with all of the environmental, pharmacological and pathological variables. Therefore, MS is essentially important technology for personalized patient management.

Significance of systemic inflammatory markers in patients with systemic diseases

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Systemic diseases are generally an interdisciplinary challenge in clinical practice. Systemic diseases are able to induce tissue damage in different organs with ongoing duration of the illness. The heart and the circulation are important targets in systemic diseases.
A wide variety of systemic diseases may affect the heart by a number of different mechanisms, including increasing demands on the heart, causing arrhythmias, affecting the structure of the heart or promoting cardiovascular disease and therefore coronary heart disease.
Coronary artery disease (CAD) also known as atherosclerotic heart disease, coronary heart disease or ischemic heart disease (IHD), has been defined as a progressive disease process that causes focal thickening of large- to medium–seized muscular and large elastic arteries. Atherosclerotic vascular diseases are the number one cause of death globally, accounting for 30% of all deaths worldwide
New scientific evidence from the last two decades including epidemiological, in vivo and in vitro assays support the notion that the immune system significantly contributes in the development and progression of atherosclerosis
This new theory proposes that any potential noxious challenge to the host immune response could be related to the pathogenesis of atherosclerosis
Traditional risk factors for atherosclerosis and consequent CAD, such as hypertension, hypercholesterolemia, diabetes mellitus, marked obesity, smoking and physical inactivity, do not account for fully half of all cases of atherosclerosis.
Inflammation and the systemic immune response are believed to play a central role in the initiation and progression of atherosclerosis.
Inflammatory response and cytokine elaboration are integral components of the host response to the tissue injury and an active role after myocardial infarction.
Elevated values of circulating inflammatory markers such as CRP, serum amyloid A, IL-6, and IL-1 receptor antagonist commonly accompany CAD. Such elevations correlate with in-hospital and short-term adverse prognosis and may reflect not only a high prevalence of myocardial necrosis, ischemia-reperfusion damage, or severe coronary atherosclerosis but also a primary inflammatory instigator of coronary instability.
The acute-phase response is a non-specific process that may occur in the initial host response to injures, infections, ischemic necrosis or malignancy. It is initiated by the activation of local macrophages and other cells leading the release of mediators such as TNF-alpha, interleukin-6 and interleukin-1 beta. These in turn cause systemic changes including hepatic release of a range of plasma proteins, including CRP, activation of complement proteins and various of metabolic changes. IL-6 also promotes induction of fibrinogen, haptogloblin, ***α1- antitrypsin and α 2 macrogloblin among others.***
The purpose of our study was to assess the serum levels of high-sensitivity C reactive protein (hs-CRP), interleukin-1 beta (IL-1***β***), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-***α***) between patients with and without coronary heart disease.
These results demonstrated that inflammatory markers are significantly higher in patients with coronary heart disease compared with healthy group, especially for hs-CRP.
CRP is the best studied of the inflammatory biomarkers in CAD. CRP is not only a powerful inflammatory marker, but increasing evidence suggests that CRP may also directly participate in the inflammatory process of atherogenesis.
Keywords: inflammatory markers, systemic diseases, CAD

Low grade inflammatory response to obese and non-obese subjects, facts and promises.

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While researched the inflammatory response induced by diet, we have come across two terminologies that describe it. Generally they are the two most commonly used as, chronic inflammation and Low-grade inflammation response, but the latter is more appropriate for conception and preferred.
It is generally known that there is a greater presence of inflammatory elements in obese individuals, but how could be readouts in term of a 'low grade of inflammation' compered to non-obese subjects ,we tried to find out in this study review.
Many inflammatory mediators are released by adipose tissue,more expressed at obese subjects. Many of inflammatory markers are present at obese people in higher concentrations of lean people do. Infiltrations of macrophages in fatty tissue of the obese people seem to be a clear relation between obesity and pro inflammatory tendency. Therefore, it is believed that many of these mediators of inflammation are the trigger of many metabolic diseases, which begin as reactions at the cellular level and until the onset of metabolic syndrome where its insulin-resistance and the appearance of diabetes are at its center.
Likewise, hours following the consumption of a meal, there is an elevation in the concentrations of inflammatory mediators in the bloodstream which is exaggerated in obese subjects and in type 2 diabetics although this is quite

difficult to differentiate when other chronic inflammatory situations occur.
Low grade inflammation response is induced mostly by high-fat, high-glucose meals and high meal content of advanced glycation end products (AGE), too. It has been proven that involvement of certain antioxidants or antioxidant-containing foods within the meal has greatly mitigated their adverse effects. The most known of healthy diet component are vegetables and fruits , whole grains , fish, PUFA(polyunsaturated fatty acids), especially long-chain n-3 PUFA, are all associated with lower inflammation or anti-inflammatory effects, while meal content AGE ,SFA(saturated fatty acids) ,trans-MUFA (monounsaturated fatty acids) are associated with inflammation and enhanced oxidative stress thus creating all the prerequisites for metabolic syndrome. The best monitoring of low grade inflammatory response is through pro-inflammatory and anti-inflammatory mediators concentrations acute-phase proteins, pro-inflammatory cytokines,chemokines, soluble adhesion molecules,adipokines (TNF,Interleucines,MCP1 ,IFN,.),etc. .It is generally accepted that a consider amount of these mediators produced are adipocytes and macrophages infiltrated therein.
Main focus in this review was to analyze the literature regarding the presence of typical inflammatory mediators in obese and healthy individuals in some in some cross-section studies, selected according to the purpose of this paper. We used sources in PubMed databases.
In general, the low grade inflammation response, although being influenced by the type of diet and the modifiers of inflammatory markers, nevertheless of their mixed effects there is a clear trend of low grade inflammation, expressed through higher values of inflammation mediators in obese versus non-obese.
Studies targeting the obese population group with a well-defined diet according to a calorie and ingredients report and identifying or excluding of inflammatory modifiers, would be the best approaches to differentiate a diet with less inflammatory effect on the obese population.
Such a diet would create all the prerequisites for a reduction in obesity as well as a reduction of low grade inflammatory response.
Keywords: Obesity, Inflammation, Metabolic syndrome, Low-grade inflammation, Postprandial inflammatory response, Pro- and anti- inflammatory mediators, Pro- and anti- inflammatory diet.

Assessment of Vitamin D status deficiency in Albanian pregnant women

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There are many evidences suggesting that vitamin D deficiency is related with mother problems during pregnancy such as pre-eclampsia, gestational diabetes mellitus, metabolic disorders, increased risk for caesarean_section and also with fetal complications such as impaired fetal growth, lower bone mineral density, respiratory infections, small size for gestational age, etc.
Serum levels of 25-hydroxyvitamin D (25-OH-D) were evaluated in 185 Albanian healthy pregnant women aged 18-47 years old, which are presented at the National Blood Transfusion Centre during the period from July to December 2018. A general information form was completed for each pregnant woman included in the study. In this form, for every pregnant woman, were collected general demographic data (self-reported) regarding age (in years), weeks of pregnancy, place of residence, number of pregnancy, education, use of multivitamins and/or vitamin D, smoking, alcohol etc. All participants with a history of chronic diseases were excluded from the study. The gestational age of the participants was a 3-41 week. 25-OH-D levels were evaluated on a blood sample obtained by venepuncture in a plain tube. Serum level of 25-OH-D was measured using the CMIA method in Abbott Architect i2000 platform. We used the Endocrine Society recommendation cut-off of 25-OH-D to define vitamin D status: <20 ng/mL deficiency; 20-30 ng/ml insufficiency; 31-50 ng/mL adequate Vitamin D status.
Of 185 Albanian pregnant women participating in our study we found that: 9 (4.9%) participants result with vitamin D severe deficiency <10 ng/mL as cut off (95%CI, 5.71-8.42 ng/mL) ; 66 (35.6%) participants result with vitamin D deficiency 10-20 ng/mL (95%CI, 14.7-16.22 ng/mL) ; 62 (33.5%) participants result with vitamin D insufficiency 20-30 ng/mL (95%CI, 24.38-25.95 ng/mL) and only 48 (26%) of them had optimal levels of vitamin D (>30 ng/mL as cut off). High percentage (74%) of pregnant women had vitamin D levels ≤30 ng / mL (75nmol / L) and only 26% had normal levels >30ng/mL (75nmol / L). It is important to note that the factors affecting vitamin D levels in our study are: Season: the prevalence of vitamin D deficiency is higher in winter (100%) and decreases towards summer (62%); Age: with age increases, the prevalence of vitamin D deficiency decreases; Gestational age: the prevalence of vitamin D deficiency is lower in the third trimester of pregnancy; Vitamin D levels increase with increasing intake of multivitamin and/or vitamin D supplements. While the least important factors resulted, engagement at work, education level, number of pregnancies.
Vitamin D deficiency in Albanian pregnant women is in significant percentage, up to 40.5 % are vitamin D deficient and 74% had vitamin D levels ≤30 ng/ml. It is necessary to elaborate a national screening and treatment strategy to detect vitamin D status, especially in high-risk groups such as pregnant women.

Anti Müllerian Hormone: New roles for an established biomarker of ovarian reserve

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Anti Müllerian Hormone (AMH) is a homodimeric glycoprotein that belongs to transforming growth factor b (TGF-b) superfamily. In females, AMH is secreted by primary, secondary, pre-antral and small antral follicles (<7 mm). Since its serum concentration is strongly correlated with the ultrasound marker antral follicle count (AFC), AMH represents a reliable biomarker of ovarian function, having also the advantage of low variation within and between cycles. In our days, AMH plays an increasing role in the forecasting of reproductive lifespan, the prediction of menopause onset, ovarian response to stimulation in ART techniques, iatrogenic amenorrhea due to ovarian surgery or gonadotoxic cancer treatment, and has also proposed as a marker of Polycystic Ovary Syndrome (PCOS).
In serum, AMH is found in different forms: an inactive non-cleaved form known as pro-AMH and a cleaved, biologically active form AMH composed by N- and C-terminal fragments. Both Pro-AMH and active AMH are detected by immunometric assays. Until recently, enzyme-immunoassays (mainly Beckman Gen II, EIA/AMH Immunotech, and Anshlab assays: Ultrasensitive (Al-105i) and Pico-AMH) were used for the determination of AMH concentrations. Since 2014, automated techniques have been developed (Roche Elecsys AMH and Beckman Coulter Access AMH) and have improved the sensitivity and reproducibility of AMH measurements showing 15% to 20% lower values compared to manual assays.
In normo-ovulatory women, a peak of AMH secretion is observed between 20-25 years of age with AMH values decline thereafter until menopause. It is estimated that 34% of total AMH variation is due to age. A recent study suggested median age-specific values of AMH for normo-ovulating women with Elecsys assays: 4/3.31/2.81/2/0.882 and 0.071 ng/mL for age ranges respectively: 20–24/25–29/30–34/35–39/40–44 and 45–50 years. Similar median AMH values were also found in our study with Roche Cobas e411: 6.7/3.9/2.3/1.6/0.84/0.11 for the same age ranges.
The estimated age of menopause is important for women seeking fertility individualized counselling, or oocyte preservation. So far, no marker enough reliable exists to assess the onset of menopause. AMH may be a more effective marker than FSH, menstrual irregularities, or maternal age alone. AMH levels decrease from 5.6% per year, and become undetectable during the 3–5 years before menopause onset. A meta-analysis showed that AMH associated to age was more effective in the prediction of early menopause than age alone. However, a specific AMH threshold for menopause is still under debate.
Treatments such as chemotherapy (CT), radiotherapy, ovarian surgery are known to have detrimental effects on female fertility. Recent studies have suggested that AMH could be used to predict ovarian follicle loss for CT patients. In a large prospective study, mean basal AMH levels were 4.19 ng/mL and 4 months after CT completion, AMH levels were of 0.78 ng/mL. Moreover, a prognostic score to estimate the time to recovery of ovarian function following chemotherapy was developed based on age, AMH and BMI.
It remains unclear whether low AMH levels are predictive of lower spontaneous fertility. A prospective study conducted on patients aged from 30 to 44 years old found lower fertility rates in patients with AMH levels under 0.7 ng/mL.

Conversely, by measuring biomarkers of ovarian reserve (AMH, FSH and Inhibin B), another study showed that women with low AMH levels (<0.7 ng/mL) did not have a significantly different predicted probability of conceiving compared to other women, after 6 or 12 cycles.
AMH appears to be a weak independent predictor of qualitative outcomes of assisted reproductive technology (ART) such as implantation, pregnancy, and live birth. Meta-analysis has shown that the predictive accuracy of AMH on live birth in women undergoing IVF was poor. Although different AMH values (from 0,3 to 1 ng/mL) have been proposed, no clear AMH threshold exists to conclude on a low, normal or increased ovarian reserve, nor on the chances of a future pregnancy.
Since AMH is associated with AFC, may be the best predictive marker of hyper or hypo-response to ovarian stimulation. Since 2013, dosing AMH before IVF is recommended by ESHRE (European Society of Human Reproduction and Embryology) and NICE (National Institute of Excellence for Health and Care) to individualize strategies for ovarian stimulation.
Concerning 5 to 10% of women, PCOS is the most common cause of chronic anovulation and hyperandrogenism in young women. Since a solid correlation exists between AMH and AFC, AMH may play a role in the diagnosis of PCOS. Its use is yet not recognized in clinical practice. In vitro, AMH production by granulosa cells was found to be 4-fold higher in normo ovulatory PCOS and 75-fold higher in anovulatory PCOS compared to normal ovaries, suggesting that AMH in PCOS women is not only explained by the increase of pre-antral and small antral follicles. However, no AMH threshold exists to define PCOS. Despite this absence, American Association of Clinical Endocrinologists proposed that AMH might be an interesting alternative, while the new ESHRE guidelines do not recommend the use of serum AMH levels as an alternative for the detection of PCOM, nor as a single test for the diagnosis of PCOS.

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Evidence of HbC disease in Albania - Clinical heterogeneity related to combination with other haemoglobin disorders

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Albania is one of the Mediterranean countries where inherited hemoglobin disorders (thalassemia and hemoglobinophaties) are considerably widespread and constitute a major concern for public health even today. Screening studies have noted a high frequency of β- thalassemia carriers in the western lowland areas. Besides the β-thalassemia, all screening studies conducted on the Albanian population have found a high presence of another hemoglobin disorder, hemoglobin S (sickle cell disease), in various areas of the country. The frequency of HbS has been found to be particularly high (up to 12%) in the central areas of the western lowland. Studies have also identified carriers of Hb O-Arab, Hb Lepore, double heterozygotes HbS/β-thalasaemia and some carriers of alpha thalasaemia.
In this presentation we report our data about the presence of haemoglobin C variant in Albanian population and we describe some of the distinctive clinical features of the disease related to the combination with other haemoglobin disorders.
A retrospective study was conducted. Data were collected from the results of the anemia screening and diagnosis unit of the Laboratory Department, University Hospital Center "Mother Teresa" between 2006 and 2018. Clinical data relating to geographical origin, place of birth, age, disease onset, comorbidity, and past and ongoing treatments were collected.
Laboratory tests were performed as part of a routine diagnostic evaluation. CBC (complete blood count) and biochemical parameters were determined by automated routine procedures. Hemoglobin electrophoresis was performed in alkaline and acid agarose gel using Hyrys Hydrasys SEBIA system.
From 2006 to 2018 we have identified 15 cases with presence of HbC. 80% of our patients were women and 20% were man. Only 1 patient was in pediatric age. The median age was 33 years (range 10-52). 14 patients were Albanian from central and south areas of western lowland. 1 patient was from Nigeria.
53% of our patients (8 cases) result with HbC trait. We have found 5 cases with Hb SC disease, 1 case with Hb C homozygote and 1 case with HbC/β+ thalassaemia. Clinical picture for our HbC trait patients was nonspecific anemia. In this group, general hematological findings didn't reveal any important or evident change. Painful crisis, acute chest syndrome, cholelithiasis with icterus, pain and fever were the main clinical features in our Hb SC disease cases. Our patient with HbC/β+ thalassaemia was followed-up for several years in the Hematology Department of our University Hospital for anemia symptoms with splenomegaly, abdominal pain crises and recurrent weakness.
The presence of HbC is a rare event in Europe and Mediterranean region where thalassemia and HbS are more frequently encountered. The rarely diagnosed cases are linked with the migration of people from West-Central Africa and their movements in the trade routes that connected these areas with Europe in centuries. The subjects found in our population do not refer any descent indicative for mutation migration. An additional reason for HbC presence in Albania might also be the past presence of malaria. Until the mid-twentieth century, malaria has been the principal medical and social cause influencing the reduction of the number of the Albanian population. This disease was endemic in western lowlands, which was the origin of the above mentioned patients.
The clinical presentation, as also confirmed in our suspected and diagnosed cases at an adult age, is discrete and unclear. HbC carriers might never be diagnosed because they are asymptomatic. The most serious clinical presentation belongs to HbC/HbS forms where sickling phenomenon might lead to pulmonary complications, cholelythiasis, retinal phenomena, osteonecrosis, etc. From morbidity and mortality point of view, HbC presence, particularly when combined with HbS or thalassemia, is problematic during gestation, especially in the perinatal period.
The correct diagnosis of HbC presence can't be confirmed with standard methods used for the screening of thalassemia and sickle cell disease in our country. In literature is emphasized that hematological changes might be absent or to a degree that is not an indication for diagnosis. The changes in the peripheral blood smear, although characteristic, do not confirm the diagnosis because they can also be encountered in other forms of hemoglobinopathy. The common electrophoresis in alkaline pH gives information only about the presence of a band that migrates in A_2 position which can be HbC, HbO-Arab or HbE. Electrophoresis at acidic pH confirms the diagnosis because not only identifies HbC but gives exact data on the relative percentage of HbC and the other fractions of the patient. In cases where it is suspected the combination of HbC with alpha or beta-thalassemia, the diagnosis confirmation can be achieved only by molecular biology methods. Chromatography is also a method of choice to correctly diagnose hemoglobinopathies including HbC presence, due to the short time of examination and comparable cost regarding to other methods.
HbC seems to be a hemoglobin variant widespreed in areas reported as endemic of hemoglobinopathies in Albania. Due to the morbidity and complications it may manifest in patients, it is necessary the application of neonatal screening programs for HbC and HbS, at least for the subjects whose origin is from the areas with high prevalence of thalassemia and hemoglobinopaties.

Analytical performance goals

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Quality laboratory results are one of the factors involved in patient safety. Discussing performance specifications has a long history of more than 70 years in the laboratory medicine because it is long realized that it is impossible, and rather non-productive, to discuss quality in laboratory medicine unless analytical quality specifications (quality goals, analytical goals, or analytical performance goals) are set a priori.
Analytical performance specifications are required for many purposes, including: 1) to assist laboratorians in choosing and evaluating new assay methods; 2) to assist the organizers of EQA/PT schemes; 3) to help the manufacturers of instruments and reagents, in design, construction and marketing; and 4) to encourage laboratories to decide which particular examinations require improvement.
First universal initiative to harmonize goal setting was reflected in the 1999 Stockholm Consensus statement in which a 5-level hierarchy was proposed. In 2014, in the Milan Congress, the Stockholm hierarchy was reduced to three models based on: 1) clinical outcome; 2) biological variation; and 3) state of the art.
Depending on how quality of performance is defined, analytical performance specifications can be presented as separate goals for bias and imprecision or as combined goals in the form of allowable total error. Total error model has the advantages of: 1) compatibility with Six Sigma concept, and 2) usability in internal and external quality control. Therefore, Even if separate goals are preferred, when it comes to QC planning and Six Sigma, allowable total errors is needed.

Quality management: illuminating the path to ISO 15189 accreditation - A view from the Republic of North Macedonia

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In the Republic of North Macedonia the work of the diagnostic medical laboratories is regulated by the Law of Health Care. There is an urgent need for better development of an evidence-based, scientific, and sustainable national strategy for the improvement of health laboratory service. Clear indicators of improvement must be established. A key indicator should be the number of laboratories that have achieved, and can maintain accreditation.
The Macedonian Society of Medical Biochemistry and Laboratory Medicine (MSMBLM) recommends that the quality system established meet the requirements of the International Standard for medical laboratories ('Medical laboratories: Requirements for quality and competence '[EN ISO 15189:2012]), which has been accepted as the fundamental standard for the accreditation of medical laboratories in European countries. EN ISO 15189 was developed as a baseline standard for the Quality Management System (QMS) in medical laboratories and is recognised as the connecting standard for all disciplines in laboratory medicine. With the acceptance of the ISO standard, the need of countries for their own QMS for laboratory medicine no longer existed.
In 2013, the Standardisation Institute of the Republic of North Macedonia accepted the standard as the Macedonian norm for quality assessment of medical laboratories (MKS EN ISO 15189:2013).
MSMBLM, as the professional society of specialists in medical biochemistry, is responsible for the translation of international guidelines into national guidelines. These guidelines have to be in agreement with the standard EN ISO 15189.
For that purpose, cooperation between MSMBLM and the National Accreditation Body (Institute for accreditation of the Republic of North Macedonia), as well as cooperation between international medical laboratory organisations, such as International Federation of Clinical Chemistry (IFCC), European Federation of Laboratory Medicine (EFLM) and international accreditation bodies, such as International Laboratory Accreditation Cooperation (ILAC) is essential.
The accreditation of Macedonian medical laboratories is not mandatory; the decision for accreditation is voluntary. Accreditation is accessible to every client submitting an accreditation application to the Institute of Accreditation, which has been a member of ILAC since 2008. In 2013, the first medical biochemistry laboratory was accredited in the country. So far, nine medical laboratories have been accredited according the MKS EN ISO 15189:2013. Four of them are public sector laboratories. Flexible scope is not yet started for the ISO 15189 accreditation process in North Macedonia. The medicalized steps, including test's selection advice and interpretation of results are not included in accreditation process.
Diagnostic laboratories of the Institute of pathology, Medical Faculty-Skopje and Research Center for Genetic Engineering and Biotechnology "Georgi D. Efremov (Macedonian Academy of Sciences and Arts) are also using ISO 17025 as additional standard.
The low number of accredited laboratories could be the result of the shortage of financial resources, poor government attention to laboratory service, the shortage of qualified personnel and/or the lack of a national laboratory policy.
The experiences of laboratory professionals from accredited laboratories, who have a high level of knowledge, skills, and competence, are crucially important to the process of developing a competent laboratory service within the national health system.
The implementation of the Laboratory Quality Management system (LQMS) requires support of laboratories by the MSMBLM and close collaboration between specialists in laboratory medicine (medical biochemistry), technical assessors, and consultants. Each of them will give a different perspective on what should be prioritised. Implementation of a QMS should be a stepwise process but it is necessary to start with changes that can be easily accomplished and have the biggest impact. All quality essentials must be addressed. Appropriate laboratory facilities, infrastructure, and equipment for each laboratory tier level are essential to enable safely and efficient performance. Strong programs supporting quality assurance, quality control, and quality improvement should exist. They are fundamental for the establishment, maintenance and improvement of laboratory quality systems. SOPs must be well-written, understood, and implemented; laboratory personnel should routinely perform IQCs; and laboratories must be required to participate in EQA or proficiency testing (PT) programmes.
Future directions:
The globalisation of markets and migration of health professionals requires improving the laboratory diagnostic process. A quality laboratory system is the foundation of a strong national health system. Laboratory workforce, infrastructure, and quality management system are vital for the delivery of quality laboratory services. Coordination with the Ministries of Education and Health is essential for maintaining standards of education and levels of knowledge. The competency of laboratory professionals has to be maintained through mandatory participation in continuous medical education (CME).
For Government, Ministry of Health, professional association(s) and stakeholders, accreditation of medical laboratories according to ISO 15189:2012 should be a high priority. They should act together and undertake coordinated efforts to integrate accreditation programs into national health policy, planning, and health development programmes.
Key words: accreditation, ISO 15189, Quality Management System

Quality Control in Research Laboratories: Perspectives on Standardization

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Basic or applied research is based on scientific assessments aimed at unraveling new facts using new inventions and/or innovations of techniques. The quality control (QC) in the routine analysis in clinical laboratories is well established. For example, staff training and ongoing competency, maintenance of equipment, written document control, and method validation/verification are some of the important requirements in clinical laboratories. However, in research laboratories the culture regarding quality is immature although the resulting data is substantial. There are limited specific standards for research laboratories (1) and implementing the existing standards is difficult due to the peculiar characteristics of research laboratories. In a typical research laboratory, quality management systems are most commonly not a priority, the professionals' performances are measured on the publications and teaching activities and most of the staff in research laboratories are temporary (graduate students and visiting scientists). In addition, the costs of quality assurance (QA) might cause a significant loss of research time. All the mentioned issues and many other peculiar features of research laboratories impede the execution of potential quality management systems at research laboratories.
Despite the problems stated above, there is extensive interest in setting up a concept

for QC in research laboratories since it has become increasingly substantial that the researchers conduct experiments at the highest standards. In the late 1990s, it became recognized that researchers were in need of practical guidance about the best way to implement existing QA applications to non-routine analytical work. Therefore, a guide was produced by a EUROCHEM working party in order to promote QA applications in research and development and non-routine analysis (2). According to the guide, basic measurements are conducted in accordance with the Valid Analytical Measurement (VAM) (3) and supported by technical and operational quality elements. The EUROCHEM's guide advises controls at organizational, technical, and analytical levels (2). The research laboratories that implemented QA applications based on EUROCHEM's guide, had indicated some critical factors for achieving success in research laboratories. For example, it was suggested that the QA documentation system should be simple, QA system should add value to the organization, and be self-sustainable in order to keep the maintenance of the QA system due to the presence of temporary staff (graduate students and visiting scientists) (1).

As expected, every research process has its own characteristics based on the targeted objectives and experimental design. Still, the quality in the research process can be divided into three common features that represent key quality aspects; namely the quality of the objective, quality of the research approach to reach that specific objective, and the quality of the results (4). For example, the quality of the objective can be judged by a funding agency according to the research proposal in view of the aims, scientific interests, and approaches. Similarly, the quality of the results can be evaluated in panels or assessed in refereed high-impact journals. Besides, the assessment of the research approach quality is dependent on scientific and technical competence, and the presence of a quality system. Importantly, the implementation of QA systems requires a certain degree of flexibility, in which the limits determined by standards, in order to reach success in research laboratories. The need for flexibility arises from the inherent nature of research since observations and approaches in research processes cannot be defined and predicted precisely.

In order to assure effective QC in research laboratories, it is also substantial to embrace pre-analytical, analytical, and post-analytical phases just like routine measurements in clinical laboratories. In typical basic research practice, the storage conditions, the sampling time of the biological material, and sample preparation methodologies are major pre-analytical aspect. The analytical phase consists of the analytical process itself and other related approaches to obtain an analytical result. In an analytical perspective, the validation of the standard operating procedures (SOPs) and protocols represents an important issue in research laboratories in order to obtain repeatable results. Using a standardized workflow including pre-analytical, analytical, and post-analytical aspects will increase the reliability of the data produced in research laboratories.

Research laboratories have intrinsic quality criteria, namely reliability of data, reproducibility of methods, and monitorability of the research process (5), and mainly act upon it. However, what is new and necessary in research laboratories is to construct structured and pre-planned QA systems. Therefore, there is a need for developing specific QA systems for research consisting of national and international standards (2). It should be further noted that the acceptance and commitment to the potential QA systems are the initial steps for success. These systems need to be embedded in an organization's culture and therefore; it should be taught early at the undergraduate and postgraduate levels in universities.

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Bias in clinical chemistry

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"*Error*" of a single measurement result consists of random and systematic components. The "error" may be determined by comparison with the result of a reference measurement procedure or by participating in proficiency testing, but neither the systematic nor random error can be elucidated as such from a single measurement result. The average of repeated measurements is needed for estimating bias.

A qualitative concept measurement *trueness* is the "closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value". It is quantitatively expressed as *bias*. Another qualitative concept measurement *accuracy* describes the "closeness of agreement between a measured quantity value and a true quantity value of a measurand. It includes both systematic and random error components. A more accurate result has a smaller measurement error. It is on the average more true when the bias is small and more precise when the random error is small. Precision is expressed quantitatively as its opposite – *imprecision* using the unit standard deviation or relative standard deviation (e.g. %CV).

The *reasons for bias in clinical chemistry* are numerous and vary between measurement methods e.g.:

· Bias when taking samples, e.g. when samples are sometimes taken when the patient has been walking around and sometimes when he/she has been lying down. When the regulatory systems of the body adapt to gravity, the blood plasma volume is reduced to about 10% from a lying to a standing position thus increasing the concentration of macromolecules and cells in the blood of the patient.

· Instability of the sample during transport or storage, e.g. during transport in extremes of heat and cold and mechanical effects on cells and blood gases when transporting samples through pneumatic tubes in hospital transport systems.

· Uncorrected loss of measurand at extraction e.g. when preparing samples for measurement using high-performance liquid chromatography or mass-spectrometry.

· Errors when the calibrator is prepared, including errors in volume measurements or in weighing of calibrators in the laboratory

· Using sample matrix which differs from the matrix in the samples e.g. using de-fatted and lyophilized stable materials for internal quality control or proficiency testing programs.

· Interferences in the samples, e.g. the color of hemoglobin and bilirubin in hemolytic and icteric samples or the presence of high concentrations of proteins or lipids in the sample (myeloma or hyperlipidemia)

· The presence of molecules which specifically interfere with the reagents used in the measurement process, e.g. heterophilic antibodies (e.g. human antibodies against mouse IgG)

· Specificity for different epitopes in macromolecules of antibodies used in immunochemical measurement methods e.g. when measuring macromolecules including prostate- specific antigen, troponins and protein- or peptide hormones.

Metrological *traceability* is a property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty. Traceability is crucial for standardization of measurement results and for minimizing bias. A crucial and frequently underestimated factor in achieving traceability is *commutability*. Commutability is a property of a reference material that expresses the closeness of agreement between results for the reference material and results for patient samples when measured by two or more measurement procedures.

Lack of commutability is commonly due to m*atrix effects* which are the combined effects on the measurement results of all other molecules than the ones you intend to measure.

Automation has substantially reduced *repeatability imprecision* when measuring patient samples in clinical chemistry. *Reproducibility imprecision* has not been reduced to the same extent probably since it is more challenging manufacturers to improve reproducibility.

The *Joint Committee for Traceability in Laboratory Medicine* (JCTLM, http://www.jctlm.org/) was established in 2002 in response to the implementation of the European Community Directive on in vitro medical devices. Its founding organizations are the International Committee of Weights and Mesures (CIPM), the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC), and the International Laboratory Accreditation Cooperation (ILAC). The JCTLM publishes list of higher order reference materials, reference methods and reference laboratories. They are joined in this effort by other corresponding organizations including the FDA, National Metrological Institutes (NMI) etc. in other parts of the world. Though far from easy, through perseverance we are likely to see a bountiful harvest of the work done by JCTLM, especially as producers of reagents and systems and organizers of proficiency testing programs increasingly adopt the facilities that JCTLM brings together.

The American Association of Clinical Chemistry (AACC) in 2010 initiated the *International Consortium for Harmonization of Clinical Laboratory Results* (ICHCLR, https://www.harmonization.net) organizing a global effort to

harmonize test results especially in the instances where standardisation is not feasible. Amongst the activities of the consortium is the publication of a toolbox of approaches and procedures to be used when developing a process to achieve harmonization for a measurand.
Further developments in reference measurement systems is likely continue to play the major role in minimizing bias in clinical chemistry in the decade ahead. Reference measurement systems are, however, unlikely to solve the most complex bias issues, e.g. in the fields of immunochemistry. Natural patient samples are commutable and in abundant supply in the laboratories of clinical chemistry. They represent an asset that is likely to be increasingly used for minimizing bias using harmonisation methods which promise to minimize bias and measurement uncertainty in clinical chemistry still further.

Value and impact of the clinical laboratory in healthcare

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Laboratory medicine is the branch of medicine that provides objective data to clinicians and other healthcare workers to guide appropriate clinical decision making. Laboratory medicine is integral to many clinical decisions on prevention, diagnosis, treatment, and disease management (CLB 2017). It supplies health care professionals with evidence-based data necessary to provide high-quality, safe, effective and appropriate care to patients. Unfortunately, this critical role of laboratory medicine is not widely recognized within healthcare organizations, leading to poor visibility both within the field of clinical medicine and externally with the public at large. The laboratory is viewed as a black box where patient specimens are sent and test results are magically generated. There is very little understanding of the laboratory testing process not only with patients but also physicians and other healthcare workers. This is in large part due to the low visibility of the important work carried out in clinical laboratories and the poor recognition of the major developments in laboratory testing technology that have contributed to an increasingly vital role in evidence-based clinical decision making.
Systematic evidence for the contribution of the clinical laboratory to the overall assessment, diagnosis, and management of patients is not readily available. Establishing this evidence is vital to all promotional activities by the IFCC and other organizations involved in laboratory medicine. There is a critical need for both a systematic review of the available evidence in the published literature as well as the initiation of new retrospective and prospective studies to more clearly establish this crucial evidence. The IFCC established a new taskforce to evaluate the published evidence on value and impact of laboratory medicine on clinical outcomes and healthcare delivery, and if necessary propose new studies to more clearly establish this evidence. I will review the evidence supporting the key role of laboratory medicine in clinical management and outcomes and identify the gaps requiring new studies. This will be followed by a discussion of data demonstrating the value of laboratory testing from a clinical and economical perspective. I will also review the key activities of the IFCC in promoting the visibility of the field of laboratory medicine among healthcare professionals, hospital administrators, governmental regulators and funders, and the general public.

Lipid guidelines: emerging evidence on importance of non-fasting and postprandial lipids

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With the current eating patterns in Western societies, the fed state predominates over the course of a day, with the typical individual only in the fasted state for a few hours in the early morning. Nevertheless, the fasting lipid profile has been a standard assessment of cardiovascular disease (CVD) risk. There are two primary reasons for traditionally measuring fasting triglycerides (TG): to reduce the variability in TG concentration following meal ingestion and to accurately calculate low-density lipoprotein cholesterol (LDL-C) using the Friedewald equation. However, nonfasting (i.e. random blood sample measurement irrespective of time since last meal) TG levels have been reported to fluctuate only modestly within the same individual. Additionally, calculated LDL-C has been shown to change minimally after food intake and measured and calculated LDL-C are highly correlated between fasting and nonfasting state. As nonfasting TG levels are independently associated with cardiovascular event, a paradigm shift towards assessing lipid parameters in the nonfasting or postprandial (i.e. blood sample measurement at specified time points following a standardized meal) state is occurring. In fact, postprandial TG levels obtained after consuming a standardized high-fat meal, better predict coronary artery disease compared to fasting TG levels. Several clinical guidelines have included nonfasting lipid testing in the primary prevention setting, including Denmark in 2009, UK in 2014, as well as the European Atherosclerosis Society (EAS) and European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and the Canadian Cardiovascular Guidelines in 2016. The nonfasting lipid panel has become the clinical standard in Denmark, offering physicians the option to measure fasting lipids when TG > 4mmol/L, while in Canada it is recommended to obtain a fasting measurement when TG >4.5mmol/L. Furthermore, the EAS/EFLM guidelines state that nonfasting and fasting measurements should be complementary and not mutually exclusive. The option of nonfasting lipid testing has also been included in The 2011 National Heart, Lung, and Blood Institute (NHLBI) Guidelines specific for the pediatric population. Assessing the postprandial lipid profile can provide a better indication of an individual's capacity to metabolize lipids following a meal, reflecting their metabolic efficiency.

Apolipoprotein profiling for addressing residual cardiovascular risk: in search of a personalized and metrologically sound answer to the latest dyslipidaemia guidelines

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An elevated low-density lipoprotein cholesterol (LDLc) concentration is a classical risk factor for cardiovascular disease. This has led to pharmacotherapy in patients with atherosclerotic heart disease or high heart disease risk with statins to reduce serum LDLc. Even in patients in whom the target levels of LDLc are reached, there remains a significant residual cardiovascular risk; this is due, in part, to a focus on LDLc alone and neglect of other important aspects of lipoprotein metabolism. According to the latest dyslipidaemia guidelines, a more refined lipoprotein analysis is advocated, especially for secondary prevention, which provides additional information on the accumulation of very low-density lipoproteins, intermediate density lipoproteins, chylomicrons, chylomicron-remnants and Lp(a). Instead of measuring the overall cholesterol and triglyceride content of lipoproteins, measurement of their apolipoproteins is more informative. Apolipoproteins are either specific for a particular lipoprotein or for a group of lipoproteins. Measurement of apolipoproteins in atherogenic particles is more biologically meaningful than the measurement of the cholesterol concentration contained in these particles. Applying serum apolipoprotein profiling will not only improve characterization of lipoprotein abnormalities, but will also improve definition of therapeutic targets. Apolipoprotein profiling aligns with the concept of precision medicine by which an individual patient is not treated as 'average' patient by the average (dose of) therapy. This concept of precision medicine fits the unmet clinical need for stratified cardiovascular medicine. The requirements for clinical application of proteomics, including apolipoprotein profiling, can now be met using robust mass spectrometry technology which offers desirable analytical performance and standardization.
Keywords: Dyslipidaemia, mass spectrometry, clinical proteomics, metrological traceability, serum apolipoprotein profiling

The importance of cholesterol synthesis and absorption markers determination in healthy subjects and patients with ischemic heart disease

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According to the World Health Organization, worldwide incidence of cardiovascular diseases (CVDs) is everincreasing, and CVD are among the leading causes of morbidity, co-morbidity and mortality. Atherosclerosis is a chronic, focal disease of the blood vessel intima. Atherosclerosis is the underlying

cause of the most CVD, including coronary artery disease (CAD). Even though many etiological factors are involved in the pathogenesis and progression of atherosclerosis, dyslipidemia has the key role in atheroma development. Statins represent a hypolipemics of choice in primary and secondary CAD prevention. In addition to the inhibitory effect on cholesterol synthesis, statins also have numerous pleiotropic effects. Basic lipid parameters are used for diagnosing dyslipidemia and monitoring the statin therapy response in clinical practice. Elevated plasma total cholesterol (TC), LDL-cholesterol (LDL-C) and triglyceride (TG) concentrations and low HDL-cholesterol (HDL-C) concentrations represent well-documented risk factors for CVD. However, in order to examine the overall cholesterol metabolism and monitor its homeostasis, it is necessary to examine the efficiency of cholesterol synthesis and absorption, its distribution between lipoprotein particles, and the preservation of the reverse cholesterol transport function. Cholesterol homeostasis represents the balance between cholesterol synthesis and absorption. Many studies have shown that cholesterol synthesis and absorption are in equilibrium. Increased cholesterol synthesis leads to reduced absorption and vice versa, in order to maintain balance. Cholesterol synthesis is divided into two different pathways, that may be independently regulated (80% via the lathosterol - Kandutsch-Russel pathway; 20% via the desmosterol - Bloch pathway). Non-cholesterol sterols (NCSs) represent cholesterol synthesis precursors (desmosterol and lathosterol) and cholesterol absorption surrogate markers (phytosterols - campesterol, stigmasterol and β-sitosterol). Knowing that the plant sterols are absorbed in the same way as the intestinal cholesterol, plant sterols are used as surrogate markers of cholesterol absorption efficiency. These markers can indicate early development of dyslipidemia and predict response to statin therapy. NCSs concentrations in plasma are 200–1000 times lower compared to cholesterol levels and relatively low NCSs concentrations represent a specific problem for their quantification. This represents the additional reason to conduct an extensive method validation for NCSs determination, as well as to resolve pre-analytical and analytical factors of influence. In order to contribute to a better understanding of cholesterol metabolism and the statin effects on cholesterol homeostasis, the objectives of this study were: establishing and validating the method for NHSs determination; determination of NHSs concentrations in healthy subjects (CG) and CAD patients; determination of cholesterol homeostasis patterns and their association with basic lipid parameters and distribution of low-density lipoprotein subclasses (LDL) in examined groups. The study included 31 healthy controls (CG), 32 statin-treated patients and 47 statin-naive CAD patients. Method optimization, validation and stability studies were executed in human serum and plasma. Freeze-thaw cycles were done with and without antioxidant. Gas chromatography-mass spectrometer (GC-MS) was used for NCSs confirmation and plasticizer identification, while GC-flame ionization detector (GC-FID) was used for NCSs quantitation. Lipoprotein subclasses were separated by gradient gel electrophoresis (GGE).

The results of this study have shown that both serum and plasma are adequate biological materials for NCSs determination. Intra- and inter-assay variabilities for all NCSs were 2.75–9.55% and 5.80–7.75% for plasma and 3.10–5.72% and 3.05–10.92% for serum, respectively. Recovery studies showed satisfactory percentage errors for all NCSs: 93.4–105.7% in plasma and 87.5–106.9 in serum. The presented results showed that the derivatization of samples is necessary in order to obtain adequate chromatographic NCSs separation. Derivatized samples were stable up to 7 days at −20 °C and derivatization yield was a□ected by presence of plasticizers. Fatty acid amides were identified as interfering plastic leachates and our results shown that the use of plastic laboratory consumables should be avoided in NCSs analysis. Statistically, di□erent NCSs concentrations were observed after the 1st freeze-thaw cycle, in antioxidant-free samples, and after the 4th cycle in antioxidant-enriched samples. The concentrations of desmosterol and lathosterol were significantly higher in both groups of patients compared to CG. Desmosterol absolute values were higher in patients receiving no statin treatment compared to patients with statin treatment and controls. Desmosterol concentrations showed a negative correlation with the LDL particle size in CG ($r = -0.459$, $p = 0.016$) and in a statin-naive patients ($r = -0.381$, $p = 0.012$). For the assessment of cholesterol homeostasis, we divided each group of participants into four subgroups with good or poor synthesis and good or poor absorption (PS/PA, PS/GA, GS/PA and GS/GA) according to desmosterol or lathosterol and β-sitosterol median values. Within subgroups, total cholesterol levels increased with increasing synthesis and/or absorption. In CG, the GS/PA subgroup had the highest triglyceride values and the largest proportion of small dense LDL particles. In the statin-treated patients, GS/PA subgroup had the lowest LDL-cholesterol concentration and the smallest LDL IVB subclasses distribution compared to other groups.

Derivatization, as well as derivatization yield assessment, was shown to be necessary in order to accomplish the reliable quantitation of the cholesterol precursors. Also, when applying derivatization, special care must be taken during the selection of appropriate labware and laboratory consumables. Based on NHSs concentrations, it is possible to determinate cholesterol synthesis and absorption patterns and identify individuals at high risk for CVD development and progression. In addition, determinations of cholesterol homeostasis patterns are potentially useful tool for predicting the individual propensity toward hypolipidemic therapy response.

Key words: dyslipidemia, non-cholesterol sterols, cholesterol homeostasis, plasticizer

Impact of redox imbalance and inflammation on activity of paraoxonase 1 and its distribution in high density lipoprotein in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. It is a complex endocrinological condition because of its heterogeneity, inconsistency regarding the etiology and difficulty in making the diagnosis. Over the years, diagnostic criteria for PCOS have changed. In our study, PCOS was defined on the basis of the revised Rotterdam Consensus Criteria (2003) by meeting two of the following three criteria: moderate oligo/amenorrhea (less than eight menstrual cycles per year), presence of clinical hyperandogenism (hirsutism) and/or biochemical hypeandrogenemia and the existence of polycistic ovaries confirmed by transvaginal ultrasonography.

Oxidative stress (OS) is characterised by irreversible damage of practically all cellular components (proteins, lipids and DNA) leading to impaired cell function. OS is an important link in the pathogenesis of PCOS, but it has not yet been determined whether increased levels of OS in PCOS are due to the syndrome itself or related to its characteristics (hyperandrogenism, insulin resistance (IR), obesity and abdominal obesity that significantly contribute to OS development). Chronic low-grade inflammation is an important feature of PCOS (participates in its pathogenesis and development). Numerous evidence supports the concept of feedback formation, where inflammation induces reactive oxygen species (ROS) formation, while OS exacerbates inflammation as described in endothelium and adipose tissue.

High-density lipoprotein (HDL) particles are present in the circulation in the form of different subclasses that differ in size, density and lipid composition. The antioxidant/anti-inflammatory role of HDL depends on the presence of antioxidant enzymes. Paraoxonase 1 (PON1) is an antioxidant enzyme associated with apolipoprotein A1 on HDL particles whose activity and concentration may be reduced in OS, further increasing the risk of developing cardiovascular disease (CVD). Although there are different opinions about the PON1 distribution between HDL 2 and HDL 3 subclasses, PON1 is assumed to follow the reverse cholesterol transport.

The study included 114 PCOS patients and 50 healthy females (control group, CG), of similar age (18 – 39 years). The CG participants had normal glucose metabolism, were non-smokers and zero alcohol consumers with no signs of hyperandrogenism. Patients were analysed during the early follicular phase of the menstrual cycle, or at any time if they had severe oligomenorrhea or amenorrhea. Systolic and diastolic arterial blood pressure (SBP and DBP), anthropometric, biochemical and oxidative stress parameters were determined in all study paticipants using standardised assays. Plasma HDL particles were separated using a non-denaturing 3-31% polyacrylamide gradient gel electrophoresis method, previously described by Rainwater et al. which was optimized in the laboratory of the Department of Medical Biochemistry, Faculty of Pharmacy, Belgrade. Following HDL lipoprotein electrophoresis, PON1 activity on HDL 2 and HDL 3 subclasses was determined using the Trinder reaction according to Gugliucci et al.

As we wished to examine the mutual effect of the most important risk factors in PCOS patients, we calculated the DOI score as a sum of dyslipidemia, OS and inflammatory scores.

High-density lipoprotein cholesterol (HDL-C) was significantly lower in patients compared to healthy controls ($P<0.01$) with no significant differences in triglyceride (TG), non-HDL-C concentration and atherogenic index (TG / HDL-C) values. Total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations were not elevated in women with PCOS. In contrast PCOS patients had significantly higher CRP ($P<0.001$). More pronounced oxidative stress markers such as AOPP, TOS, MDA ($P<0.001$) and PAB ($P<0.05$)

combined with a concomitant reduction in the concentration of total SH-groups and lower PON1 antioxidant activity (P<0.001, P<0.05, respectively) were found in patients compared to healthy controls. All individual scores: oxy-score (P<0.001), dyslipidemia (P<0.05) and inflammation (P<0.001) scores were significantly higher in patients compared to healthy controls. Consequently, the DOI score was significantly higher in comparison to healthy controls (P<0.001), highlighting the significance of this result for assessing cardiometabolic risk in patients.
The analysis of HDL size and HDL subclasses (HDL 2 and HDL 3) distribution showed that HDL particle size did not differ between patients and healthy controls. However, normal weight patients had significantly higher HDL 2 subclass than normal weight and obese controls (P<0.05). HDL particle size analysis within the PCOS group showed that obese patients had significantly smaller HDL diameters (P<0.05) and a greater HDL 3 subclass than normal weight patients (P<0.05). This was consistent with previous findings that showed a decrease in HDL particle size in patients with higher CVD risk.
PON1 distribution within HDL subclasses did not differ between patients and healthy controls. Obesity had no influence on PON1 distribution within HDL subclasses in patients. However, in patients with small LDL particle size the relative proportion of PON1 on HDL 2 subclasses was significantly higher (P<0.001) and the relative proportion of PON1 on HDL 3 subclasses was significantly lower (P<0.01) than in patients with large LDL particle size. As patients had a higher antioxidant score, the relative proportion of PON1 on HDL 2 subclasses increased (P<0.01) while the total proportion of PON1 in the HDL 3 subclasses decreased (P<0.05). Increased oxy-score was accompanied by an increase in the total proportion of PON1 on HDL 3 subclasses (P<0.05).
The results demonstrate that PCOS women have elevated levels of OS markers i.e. products of their action, and decreased values of antioxidant protection parameters. Patients have higher OS, dyslipidemia as well as chronic low-grade inflammation compared to healthy women that indicates currently low cardiovascular risk. Obese PCOS patients have significantly smaller HDL diameters and a higher proportion of small HDL 3 subclasses (associated with a high risk for CVD) compared to normal weight PCOS patients. Based on this, we can assume that obesity in PCOS affects the profile of HDL subclasses, while PCOS itself has no effect on the HDL subclasses profile. A comparison of PCOS women, according to the LDL particle size, indicated that PON1 activity on small HDL 3 subclasses was significantly lower in women with small, denser LDL particles, which is a sign that the antioxidant ability of HDL 3 subclasses is decreased in PCOS in conditions of increased risk for CVD.

Sensitive assessment of white blood cell functionality by novel hematological parameters

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Cellular and morphological analysis is an integral part of modern haematology analyzers. A unique combination of techniques permits to separate cell populations based on lipid composition of cell membranes, the fluorochrome RNA labels, cell volume and intracellular structure. In addition, the DNA of the nucleus is labeled by the fluorescence reagent penetration. The intensity of the fluorescence signal is directly proportional to the nucleic acid content and the strongest signals are shown by immature and activated cells. Sensitive assessment of cell functionality or activation status depends on cholesterol- and glycosphingolipid raft in the plasma membranes that play important roles in protein trafficking and cellular signalling. The information about membrane lipid rafts and cytoplasmic RNA is analyzed with proprietary algorithms that deliver sensitive detection of reactive or pathological cells in a blood sample. Modern hematology analyzers are designed with improved gating and optimization of leukocyte clusters including immature granulocytes (IGs). Moreover, results including the presence and concentration of IGs become available within minutes – and are included in the complete CBC+DIFF analysis, making it a valuable sixth subpopulation of the white blood cells. The measurement of the immature cells, which combine promyelocytes, myelocytes and metamyelocytes, is considered clinically useful for the diagnosis of infections, especially neonatal sepsis, inflammation, myeloproliferative diseases, tissue necrosis and acute transplant rejection at a very early stage. The results were excellent considering the low levels of IGs observed and the well known limitations of manual differentials and rare cell events. Modern analyzers are much more sensitive than the manual differential counting method in the detection of leukemic blasts and they provide more cell population data than the manual differential count, including blast lineages. Distribution and appearance of lymphocyte population quantify the numbers of all reactive, antibody-synthesizing and malignant lymphocytes. For instance, several studies showed that lymphocyte RE-LYMP and AS-LYMP counts (Sysmex XN series) were mainly increased in viral infections. Monocytic population provides information for screening and differential diagnosis of malaria and dengue by a calculated malaria factor. The modern analyzers are characterized with high sensitivity and specificity for detecting atypical cells in the samples and smear reviews can be reduced by approximately 20 % in routine laboratory. The possibilities of current haematology analyzers for better screening, diagnosis and monitoring of reactive and malignant diseases are increased without the need for clinically irrelevant follow-up tests.

The future of Cytometry in Europe

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The field of Cytometry is a recent discipline which emerged through developments in the fields of Physics (optics and fluidics) and Computer Science (electronics and informatics). Flow cytometry is today an established field that deals with the quantification of cellular characteristics. To envision the future of the field of cytometry in Europe we navigate through the past and the present in an effort to describe the state of the art on the field, based on the recent advances.
From the dawn of Cytometry during the mid-20th century till today many pioneer European cytometrists have largely contributed to the field. Among them, Wolfgang Göhde is considered as the founder of European cytometry and is the one that developed the first fluorescence-based cytometer. The field has further evolved after Kholer and Milstein (Nobel prize in Physiology or Medicine in 1984) described the development of monoclonal antibodies. During this period Flow Cytometry attracted many pioneer scientists from diverse disciplines and scientific backgrounds, including: Claude Curties, who applied cytometry in oceanographic studies and in discovery of eukaryotic organisms; Andrew Ridell, founder of the European Cytometry Network; Gerd Schmitz, founder of EWGCCA (European Working Group on Clinical Cell analysis); Günter Vallet, Pioneer of multiparametric flow cytometric analysis and one of the earliest promoters and appliers of the current concept of Cytomics and its application to Predictive Medicine; Phillip Sansonetty, organizer of the First International Workshop on Flow and Image Cytometry.
The continuous application of novel methodologies widened the scope and target audience of Flow Cytometry in both clinical and research laboratories. Such developments led to initiatives that resulted in the formation of organized Working Groups and Societies in individual Countries and in Europe as a whole. First, ISAC (International Society for Advancement of Cytometry) held many of its meetings in European countries, while many presidents of ISAC were also of European origin. Second, National Societies have been established in European Countries and a Network of Quality Control has been working since 1989. Third, a European Cytometry Network has been recently established, as an initiative of European Molecular Biology Laboratory EMBL, Heidelberg, 2008, in order to establish communication, cooperation, education and promotion of Cytometric science and techniques among its members. The European Cytometry Network (ECN) has been created with the aim to support modern infrastructure and to build up connections between professionals in Cytometry. Euroflow Network is also an established network in the field of HematoOncology, by J.J.M. Van Dongen (chair) and Alberto Orfao (co-chair), including 19 diagnostic research groups and one SME, with a vision to connect experts in the field of flow cytometry and molecular diagnostics. The European Working Group on Clinical Cell Analysis (EWGCCA) established cooperation and training in Cytometry since 1995, including the First European Course in Clinical Cytometry in Athens in 2005. As a continuation of EWGCCA activities, European Society for Clinical Cell Analysis has been established as a scientific society in 2006. ESCCA holds Annual Conferences in collaboration with local societies, annual EuroCourses (Education programs), Schools on cytometry (winter, summer, autumn), flow events, harmonization and guidelines projects. More recently ESCCA provides Certification exams for Cytometry operators and Cytometry specialists and also ESCCAbase, an organized database of flow cytometry results and analyses.
The development of European and local societies, along with the organization of Conferences and educational courses have been necessary, based on the rapid recent developments of technology and methodologies. During recent years

Multiparametric Flow Cytometry made it possible for more cell characteristics to be examined from the same cell population leading to the establishment of Cytomics. Recent developments in the field also include: Mass Cytometry, a combination of flow cytometry and mass spectrometry to interrogate up to 100 parameters from a single cell; Imaging Flow Cytometry, Using a microscope that also analyses cell shape and the relative position of different epitopes (allowing for colocalization analysis); Spectral Flow Cytometry, where newly developed multichannel detectors allow for the simultaneous analysis of more parameters minimizing the problems of spectral overlap.
The state of the art in the field include Cytometry methodologies that are among the standard techniques practiced in both clinical and research laboratories. The combined efforts of cytometrists throughout Europe guaranteed the advancement of the field up until today. Knowledge and data dissemination are critical parameters in the era of information and informatics. Our suggestion is that the future of the field should be based on cooperation, openness in knowledge sharing and organized cytometry courses for educating the new generation of cytometrists.

Significance of the determination of biomarkers of bone resorption and formation in patients with end stage renal disease

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End stage renal disease (ESRD) is associated with various mineral and bone disorders. Guidelines for improving the quality of life and education of patients with ESRD (Kidney Disease Outcomes Quality Initiatives, KDOQI), published by the U.S. National Kidney Foundation (NKF), indicate the importance of biomarkers of bone metabolism that should be analyzed in ESRD patients. Routine parameters that are determined in most laboratories are indirect indicators of bone turnover, such as: calcium ions (Ca), inorganic phosphate ions (P), magnesium ions (Mg), the total alkaline phosphatase (ALP), an intact parathyroid hormone (iPTH) and 25-hydroxy vitamin D (25D). On the other hand, direct indicators of bone metabolism are products of bone cells. The activity of osteoblasts, the cells responsible for bone formation, is well expressed by the levels of bone alkaline phosphatase isoenzymes (BALP), which is highly specific for bone tissue. The activity of osteoclasts, the cells responsible for bone resorption, specifically reflect levels of tartrate resistant acid phosphatase (TRAP). A good marker of bone resorption is the beta-carboxy terminal telopeptide of collagen type I, beta-CrossLaps (beta-CTx). The aim of this study was to evaluate the usefulness of biomarkers of bone resorption and bone formation in ESRD patients. The study included 40 predialysis patients (18 women and 22 men) aged 25−79, 114 patients on continuous ambulatory peritoneal dialysis (CAPD) (49 women and 65 men) aged 30−84 and 112 patients on hemodialysis (HD) (53 women and 59 men) aged 25−79. Average duration of the HD and CAPD treatment was 76 and 35 months, respectively. The analyzed biomarkers of bone formation and resorption were determined in the serum of patients on the day of sampling: for predialysis and CAPD patients when they came to the routine check-ups, and for HD patients immediately before dialysis therapy. To determine the reference intervals, analyzed biomarkers were measured in a group of 50 healthy volunteers (25 women and 25 men) aged 20−70 years. ALP, TRAP, Ca, P and Mg were determined using spectrophotometry (Olympus AU2700 ISE). BALP values were determined using zone electrophoresis (SEBIA Hydrasis), beta-CTx and iPTH concentrations were determined with ECLIA (Elecsys Rosche) and 25D concentrations were determined by HPLC with reversed phase detection (HPLC ChromLineR Clinical software Version 4:20). Determination of the analyzed biomarkers is considered reliable based on the coefficients of variation (CV) obtained by precision testing in the series (CV: 0.6%−3.3%) and from day to day (CV: 1.0%−3.6%). We established the normal distribution of the values for each of the analyzed biomarkers in predialysis and dialyzed patients. There was significant impact of gender on iPTH, P and CaxP values in the all analyzed groups. However, the effect of age was observed only on the values of BALP. Duration of the dialysis had impact only on the values of ALP and BALP in HD patients and on Mg concentrations in CAPD group. BALP values were significantly lower ($P<0.001$), and beta-CTx and TRAP values were significantly higher ($P<0.05$ and $P<0.01$) in ESRD patients, compared to the control group. The effect of the dialysis, regardless of the dialysis mode, was confirmed with lower BALP values in dialysis patients compared to the predialysis patients ($P<0.05$). However, we obtained much lower beta-CTx concentrations in HD patients as compared to predialysis patients ($P<0.05$). The most significant change considering the iPTH concentrations (<150 pg/mL, 150−300 pg/mL and >300 pg/mL) was observed in the BALP values in all three groups of patients. There were parallel changes in the values of BALP and iPTH in all three groups of the patients. There was significant difference in the BALP values, regarding the 25D concentrations (<50 nmol/L and >50 nmol/L) in CAPD patients ($P<0.05$). In order to determine diagnostic accuracy of direct and recommended biomarkers in relation to the recommended value of the iPTH (<100 pg/mL) for detection of adynamic bone disease in ESRD patients, we performed ROC analysis. When we analyzed all three studied groups of patients and HD patients separately, we found calcium had highest diagnostic value. The areas under the curves (AUC) were significantly different in comparison with other biomarkers analyzed (AUC=0.701, P=0.0001 and AUC=0.651, P=0.007, respectively). In the group of predialysis and CAPD patients, the highest diagnostic value had BALP (AUC=0.688 and AUC=0.588), although there was a marginal significant difference with other analyzed biomarkers (p=0.058 and p=0.053). This study support other reported data, that examined biomarkers (BALP, TRAP and beta-CTx) have comparable diagnostic accuracy as well as the recommended biomarkers (Ca, P, Mg, ALP, iPTH and 25D) to determine the level of bone metabolism in ESRD patients. On the basis of our results we can conclude that bone markers, generally, may be an appropriate alternative to invasive method of bone biopsy.

CEA monitoring in colorectal carcinoma - to the limit of the guidelines and beyond

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Carcinoembryonic antigen (CEA) is a well-established serum tumor marker in colorectal cancer. It is used in preoperative prognostication, disease stage prediction, immediate post-resection assessment and , to some extend as an adjunct in treatment response monitoring. The diagnostic and screening value of CEA is definitely poor, but the pre-operative determinations of the marker for risk stratification in patients with diagnosed colorectal cancer is widely discussed in the literature and is considered to be recommended. The discussion for the preoperative testing is much more connected with the clinical value of the marker, i. e. whether the patients with higher preoperative values to receive adjuvant therapy, due to the poorer prognosis, based on these higher values of CEA or not. The disease stage prediction, based on CEA is accepted and recommended by NCCN and ASCO, but it should be mentioned that the tumor marker values must not be considered as an indication for adjuvant therapy, but only for a basis for intensive follow-up of patients in high risk of recurrence. The data for the immediate post-resection assessment of CEA is under discussion and although there are some research studies that report positive results, the value of the marker is not considered proven and is not recommended in the official guidelines. The usage of the marker in the recurrence monitoring has a proven influence on the surveillance of patients with colorectal cancer. The major role of the marker, however, is in the monitoring/follow up of patients with colorectal cancer, treated with curative intent. CEA value in follow up of those patients is addressed in multiple randomized trials, meta analyses and systemic reviews, reaching the highest evidence bases appraisal as a monitoring tool. Although comparatively straight-forward, the diagnostic assessment in patients with elevated surveillance CEA levels may vary. Generally clinical examination, imaging (CT, MRI) and endoscopy (wherever applicable) should follow a confirmed CEA rise. There is a specific group of patients, where the so called conventional work up does not show a recurrence or fails to do so. In this scenario the clear clinical question is whether the increase In CEA levels is a false positive or a true positive for recurrence. In those cases more sophisticated diagnostic work-up may be needed including FDG PET CT and possibly novel biomarkers. PET CT shows high detection rate in previously not recognized colorectal cancer recurrence with rising CEA levels and is the modality of choice in this particular situation. If appropriately performed it demonstrates detection rate as high as about 85% in rising CEA positive patients, which is in favor of predominantly malignant reasons for CEA rise or can also lead to detection of a synchronous/metachronous primaries that also produce high CEA levels. Here breast cancer, stomach and lung cancer, pancreatic cancer and mesothelioma should be mentioned. Metastatic sites that may present as high CEA and could potentially be missed by conventional imaging are mostly lymph node metastases, peritoneal spread, local recurrence, liver and other rare locations. The minority of patients with rising

CEA levels may occasionally experience a recurrence despite negative results from the extensive work-up. Close CEA levels monitoring is essential in this scenario with the results going in two directions: those with further rising CEA levels almost invariably recur while those with high but stable CEA levels rarely experience recurrence. The absolute CEA value is also important with serum levels of CEA of more than 10ng/ml being predictive of recurrence in very high proportion of patients. However one should bear in mind that CEA levels rise in a variety of benign conditions and could reach excessive absolute values without a presence of malignancy. The most often and typical benign diseases, connected with CEA elevation are chronic hepatitis, cirrhosis, chronic kidney failure, colitis, jaundice. So neither the rise alone nor the absolute value but the trend of rising is predictive of recurrence if so-far work up has failed to localize disease. Even though guidelines and official recommendations are mostly clear about the role of CEA in the monitoring of colorectal cancer patients, treated with curative intent and the consecutive conventional and high-end imaging, the management of the patients with no recurrence detected is less clear. In these cases combined assessment with Ca 19-9 may be attempted, but with the clear idea that Ca 19-9 performs suboptimal in colorectal cancer and is a subject of broad spectrum of non-specificity. Other problem is that Ca19-9 and CEA may rise simultaneously in similar benign processes which limits the use of the combination of markers as differential diagnostic tool. In the present era of molecular and genetic testing attempts are made to correlate the rising monitoring CEA levels, such as circulating tumor cells, circulating free tumor DNA (cfDNA), methylated DNA (e.g septin 9), reporter mRNA etc. Attempts in this direction have been made also in the group of CEA positive FDG negative patients. None of the tests has however reached routine clinical use.
The development of new imaging methods and tests for detecting recurrence in patients with colorectal cancer puts on discussion whether and how underestimated is in fact the positive predictive value of CEA, including the levels below 5 ng/ml and whether in patients with higher levels of the marker the oncologist should make a great effort for clearing the reason, i.e. accepting or rejecting a recurrence, or the patient should be followed up using only conventional methods till appearance and verification of clinical symptoms of recurrence.
:Although not perfect in predicting recurrence CEA is still the monitoring tool of choice when it comes to colorectal cancer patients. Patients with rising CEA levels should be chased to prove recurrence by conventional imaging, endoscopy and FDG PET CT. Those with no proof of recurrence should be followed up strictly to define any upward trend of CEA values and in case of such should be reassessed again.

Traceability in Laboratory Medicine and IVD Directives

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A high percentage of clinical decisions are based on data stemming from Laboratory Medicine (LM). This responsibility requires delivery of a high-quality service. Method calibration is a challenge. In vitro Diagnostic (IVD) companies mostly produce their 'own' calibrators, resulting often in variability between methods for the same measurand. Variability between methods may cause incorrect patient results leading to wrong diagnosis and treatment, and poor clinical outcome. Traceability requires both (certified) reference materials and reference measurement procedures (methods) in which they are used. The Joint Committee for Traceability in Laboratory Medicine (JCTLM) was formed in 2002 by the International Bureau of Weights and Measures (BIPM), IFCC and International Laboratory Accreditation Cooperation (ILAC) to enable a global response to the IVD Directives. The In Vitro Medical Devices Directive (IVDD) 98/79/EC, introduced in 1998 was not capable of regulating all new technical and medical developments. Several weaknesses in the IVDD were identified: new developments regarding genetic testing and companion diagnostic devices that are not specifically addressed in the IVDD, the need to better align with international guidelines– including a risk-based classification system–and the lack of control over high risk "in-house" tests. The new European In Vitro Diagnostic Regulation (IVDR) EU/2017/746, published in the Official Journal of the European Union on May 5, 2017, entered into force on May 25, 2017. The biggest change is the introduction of a risk-based approach to classification in combination with increased Notified Body (NB) oversight. The official transition period for full implementation is five years. The new EU regulations create a new environment for IVD companies in terms of product development, management of product lifecycle, and commercialization approach. The new EU regulations create a new environment for IVD companies in terms of product development, management of product lifecycle, and commercialization approach.

Introduction of the European Metrology Network on Traceability in Laboratory Medicine

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The new EURAMET European Metrology Networks (EMNs) were introduced with the objective to create sustainable structures in areas of strategic importance for the future development of European metrology. The network on Traceability in Laboratory Medicine (TraceLabMed) was initiated to build a coordinated infrastructure in an area that affects almost every European citizen. In vitro diagnostics based laboratory testing is fundamental to healthcare and an important factor within the EU economy. The maximum benefit of laboratory tests for patients (proper diagnosis, reduced hospital stays, less burden on health insurances, etc.) can be achieved with tests that provide accurate results irrespective of the laboratory, the test kit, and the instruments used to obtain these results. The European regulation on in vitro diagnostic medical devices (2017/746/EU) (IVDR), which came into force in May 2017, supports this task, among other measures, by requiring metrological traceability of calibrator and control material values as well as designated EU reference laboratories. The EMN TraceLabMed was established as a central point of contact for calibration and reference laboratories and IVD manufacturers to support a coherent strategy for a pan-European response to the legal requirements for in vitro diagnostics. By facilitating collaboration across Europe, the EMN TraceLabMed will help to establish the reliability and consistency of measurement results in laboratory medicine, supporting the health of European citizens. The presentation will give a brief overview of the status and activities of the EMN TraceLabMed and the strategies used to achieve progress.

Amino Acid and Organic Acid CRMs for Newborn Screening

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The Certified Reference Material (CRM) is utilized in chemical measurements as a useful tool for proving traceability of measurement result and enhances measurement quality. Organic acid and Amino acid concentrations are frequently measured for treatment and diagnosis purposes of Inborn error of metabolism (IEM) which is a permanent and inherited biochemical disorder generally caused by organic acid, amino acid metabolism distortedness. Early diagnosis of metabolic diseases is very critical and they should be evaluated through reliable screening tests. The use of CRMs is required to ensure the quality of the chemical measurements. Particularly, it is important to use CRMs, having the same chemical compositions (matrix matched CRM), for the detection of subject quantity in the mixtures (matrix), such as body fluids, containing more than one metabolite. In this way, through the use of CRM in measurements, metrological traceability chain can be ensured.:Production and the certification of the CRMs are carried out according to the technical requirements of ISO Guide 35. Quality management system based on ISO/IEC 17025 and ISO Guide 34. IDMS method was applied as primary method of measurement for the characterisation of the materialsS:Amino acid concentrations in lyophilized human plasma are certified in UME CRM 1314.
Organic acid concentrations in lyophilized urine are certified in UME CRM 1315.:Two new certified reference materials were produced and certified to be used in newborn screening tests and routine clinical measurements for 32 amino acids in human plasma and 47 organic acids in urine.
Keywords: Certified Reference Materials (CRMs), quality control, newborn screening, metabolic disorder

ID-MS based reference measurement method for small analytes: vitamin D, creatinine, glucose, cholesterol, amino acids

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Isotope Dilution Mass Spectrometry (IDMS) is a primary method capable of providing accurate and precise results directly traceable to the International System of units. IDMS is an analytical technique based on the modification of the natural isotope composition of compounds after the addition to the sample an isotopically labeled form of the analyte. In this study, the use of LC-IDMS in analysing Vitamine D, Creatine, Cholesterol, Glucose in serum and amino acids in diluted HCL is described.:Certified references materials were provided from Nist. Liquid Chromatography- Isotope Dilution Mass Spectrometry method was used for quantification.:25-hydroxy vitamin D3 in human serum were ranging from 25.84 to 37.82 ng/g with an expanded uncertainty of 1.84 to 2.71 ng/g for 25-hydroxy vitamin D3.
Creatinine in human serum were ranging from 7.47 to 7.485 µg/g with an expanded uncertainty of 7.45E-02 to 7.74E-02 µg/g.
Glucose in human serum were ranging from 1.15 to 1.16 mg/g with an expanded uncertainty of 1.23E-02 to 1.39E-02 mg/g.
Cholesterol in human serum were ranging from 2.27 to 2.31 mg/g with an expanded uncertainty of 6.19E-02 to 6.59E-02 mg/g.
Phenlalanine, Leucine, Isoleucine and Proline in diluted HCL were 482.59, 200.64, 218.98 and 47.24 µg/g with an expanded uncertainty of 7.20, 3.24, 7.00 and 1.62 µg/g respectively.:Primary method techniques are capable of providing accurate and precise results.The reliability and performance of the method was demonstrated by uncertainty budget and method validation.
Keywords: vitamin D, creatinine, glucose, cholesterol, amino acids

Reference methods for quantification of peptides & proteins: β-amyloid in CSF, hCP, oxytocin, HbA1c, insulin, hGH

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Over the past two decades there have been important developments in the diagnosis and treatment of diseases with the increase in the number of biomarker molecules. There are now many endogenous peptides and proteins used as biomarkers and/or drugs. Amyloid-beta (Aβ) peptide, oxytocin, growth hormone, C-peptide, HbA1C are just a few of them. These molecules are used for therapeutic purposes as well as reference material for the diagnosis of the disease from plasma or serum. The use of certified reference materials (CRM) and validation of the measurement methods is a technical and regulatory issue deserves close attention.:In TUBITAK UME Laboratories, the peptide impurity analysis is performed by PICAA (Peptide Impurity Corrected Amino Acid Analysis) method. PICAA analysis involves AAA Isotope Dilution Mass Spectrometry (AAA-ID-MS / MS) and the intact peptide analysis using High Resolution Liquid Chromatography MS (LC-HR-MS/MS). Impurities from the peptide content determined by intact peptide analysis are used to correct the results of AAA analysis.:An SI traceable method was developed and validated for the impurity determination of several peptides and proteins in our laboratories. The analytical run was assessed determining, linearity, within-run accuracy and carryover. Matching the acceptance criteria the Correlation coefficient (r) of the calibration curve was found more than 0.995. The accuracy of 90% of the analyzed Quality Control was between 85.0% and 115.0%.:The PICAA method is an alternative method to the Total Mass Balance method used in peptide impurity analysis and can be performed with much less peptide/protein.
Keywords: CRM, Quantification, Peptide, Protein, Traceable

Latest developments on NMR; reference method for purity determination of small analytes and peptides

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Nuclear magnetic resonance spectroscopy (NMR) is a very significant analytical method which has been routinely used by chemists for the determination of structures of compounds. Besides this, quantitative nuclear magnetic resonance spectroscopy (qNMR) has great importance in various fields, such as drug industry, manufacturing of reference materials, food analyses and metabolite determination in human body fluids. Moreover, applications of quantitative NMR involve determination of purity of a compound and amount and concentration of a sample inside a matrix. The aim of this study is to determine the purity of some small molecules by qNMR method. It is also to obtain very useful information to be used with the mass balance method for the purity determination of larger molecules such as peptides:The purity assessment of estradiol, folic acid, human C-peptide and oxytocin were done by quantitative nuclear magnetic resonance (qNMR). Internal standard purity was determined by UME CRM 1301 chloramphenicol with a certified value of 99.58 ± 0.15% (k=2 (TÜBİTAK UME, Gebze, TR) within the traceability chain. All NMR experiments were performed at 298.15 K on a Varian VNMRS 600 spectrometer operating at 599.747 MHz for proton (1H) resonance frequency equipped with a 5 mm One NMR probe using 5 mm sample tubes. The softwares VnmrJ 4.2 and MestReNova 11.0.0 were used for data acquisition and data processing, respectively.:The purity determination studies performed within CCQM comparisons have been successfully completed. The results of these comparisons were published in the Metrologia journal and in the BIPM key comparison database (kcdb.bipm.org/). The purity of folic acid and human C-peptide were reported as 909.78 ± 2.56 mg/g, and 853.15 ± 8.06 mg/g respectively by qNMR.:NMR is the unique method, which can determine, with one analysis, a small molecule, having a single proton or a peptide possessing multiple protons. In this study, the advantages of NMR as a quantitative technique are mentioned.
Keywords: qNMR traceability purity

Development of a Reference Method for Transferrin Quantification in Serum

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Iron is one of the metals that are though to be involved in development of Alzheimer's Disease (AD). Recent developments revealed that metalloproteins transport the metals to the brain across the blood-brain barrier. Hence, reliable measurement method for determination of transferrin (TRF) in body fluids is needed for investigating the influence of TRF in AD development. This study aims to develope and validate a reference method for TRF quantification in serum.:Triple species-specific HPLC isotope dilution mass spectrometry (SS-HPLC-IDMS) approach was used for determination of TRF in serum and CSF. ERM-DA470k/IFCC (IRMM) and pooled CSF sample were used for method development and the method was validated using ERM-DA470k/IFCC. Firstly, 57Fe-TRF spike was synthesized and characterized. In triple SS-HPLC-IDMS approach, two calibration blends were prepared with 56Fe-TRF solution (traceable to NIST SRM 3126a) and the synthesised 57Fe-TRF spike solution. The sample blend was prepared with 56Fe saturated ERM-DA470k/IFCC and 57Fe-TRF spike. The measurement of 56Fe/57Fe ratios in all blends were performed on HPLC-ICP-MS system using bracketing sequence. The instruments used for the measurements were Agilent 1100 Bioinert HPLC and Agilent 8000 ICP-MS Triple Quad (Agilent Technologies). MonoQ 5/50 GL column (5 x 50 mm i.d., GE Healthcare) is used for separation of TRF sialoforms.:In the validation study, 3.0% repeatability has been obtained in measurements of 5 replicate measurements of ERM-DA470k/IFCC. The trueness of the method was tested, and varying recoveries in the range of 99.8%-105.9% were obtained.:A traceable quantification method for TRF in serum was developed and validated.
Keywords: Cerebrospinal fluid, Serum, Transferrin, SS-HPLC-IDMS

A Reference method for genetic mutation quantification of KRAS

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The TÜBİTAK National Metrology Institute (UME) is a member of the International Bureau of Weights and Measures (BIPM). The aim of the Bioanalysis Laboratory is to develop primary measurement methods in the field of biometrology and life sciences, to give primary level measurement service, to produce certified reference materials and to carry out proficiency testing needed especially in our country. The aim of this study is to describe newly developed measurement methods with digital PCR (dPCR: Digital Polymerase Chain Reaction) instrument which is a new technology product. Digital PCR instruments enables the calculation of DNA amount with the help of statistics by dividing single tube reaction to thousands to millions of smaller partitions. In digital PCR method, the copy number concentration of DNA is determined without using a certified reference material and it is considered as the reference DNA measurements method. Additionally, since calibration graph is not used, new measurement methods have much higher accuracy and lower uncertainty than Real Time PCR methods. As personalized medicine applications has increased, the dPCR device has also been widely used clinically in the screening of genetic variants and additionally in the detection of bacteria and viruses. In this presentation, examples of research and development studies conducted in Bioanalysis Laboratory with dPCR method will be summarized.

IFCC, C-RIDL; The current concept and future plans for reference intervals and decision limits

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Chair, The IFCC Committee on Reference Intervals and Decision Limits

From 1987 to 1991, the International Federation of Clinical Chemistry (IFCC) published a series of 6 papers, in which it was recommended that each laboratory follow defined procedures to produce its own reference intervals (Rıs). Although there were very important developments and implementations between the 1990s and 2008, the C28-A3 guideline, published in 2008 by CLSI and IFCC constituted the most significant step in the development of Rıs and updated in 2010 as EP28-A3c guideline that is still in current use. This guideline entitled 'Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory' provides the necessary steps mainly for the selection of reference individuals, pre-analytical and analytical considerations, analysis of reference values for a RI establishment study, transference and verification of the Rıs. The recommended process for defining a reference interval is the so-called "direct" approach, where subjects representing the reference population are selected and sampled and the specimen analyzed for this purpose. The concept of reference intervals is now well established and is based on including a fixed percentage of a reference population within the interval described by upper and lower reference limits.
Interest has been renewed in the topic as a result of the following regulatory initiatives in the last two decades: according to the European Directive 98/79 on in vitro diagnostic (IVD) medical devices, diagnostic kit manufacturers are obliged to supply their clients with appropriate Rıs for use with their assay platforms and reagents, and the International Organization for Standardization (ISO) 15189 standard for clinical laboratory accreditation states that each laboratory should periodically re-evaluate its own Rıs. In the present-day era of evidence-based medicine, there is still a big gap between theory and practice with respect to the application of Rıs as decision-making tools, despite the mandatory requirements. The IFCC, C-RIDL has published two papers including a protocol and comprehensive standard operating procedures (SOPs) for multicenter RI studies, with an indication of the utility of a panel of sera for the alignment of test results among laboratories in multicenter studies. In recent years, the IFCC, C-RIDL has coordinated a global multicenter study to establish Rıs and to explore sources of variations on reference values across several countries and published the results of the global study in two articles in CCA that are accessible through the IFCC website. For pediatric and geriatric Rıs, the challenges are even greater since samples from reference individuals are difficult to obtain. The alternative approaches are recommended especially in these cases in the EP28-A3c guideline. An alternative approach is the "indirect" approach where results from specimens are collected for routine purposes, which have been collected for screening, diagnostic or monitoring purposes and are used to determine the reference intervals. Last year, a review has been published in CCLM by C-RIDL on the use of indirect approaches to establish and verify Rıs from the results of routine laboratory testing. The indirect approach has some potential advantages compared with direct methods. The processes are faster, cheaper and do not involve patient inconvenience, discomfort or the risks associated with generating new patient health information. Indirect methods also use the same preanalytical and analytical techniques used for patient management and can provide very large numbers for assessment. Limitations to the indirect methods include possible effects of diseased subpopulations on the derived interval. Currently, the Committee is working on the comparison of different statistical techniques for indirect methods to establish reference intervals with the existing direct methods. Another point of discussion is the confusion which arises from Rıs and clinical decision limits (CDLs). As the two concepts are sometimes confused, there is a need to clarify the differences between these terms and to ensure they are easily distinguished, especially because CDLs have a clinical association with specific diseases and risks, thereby implying that effective clinical interventions are available. It is important to note that, because population-based Rıs are derived from the range of values expected in a typical community population, laboratory results that fall outside a RI do not necessarily indicate a disease but rather that additional medical follow-up and/or treatment may be warranted. In contrast, CDLs are associated with a risk of specific adverse outcomes and are commonly used to interpret laboratory test results, including lipid parameters, glucose, hemoglobin A1c (HbA1c), and tumor markers, to determine the risk of disease, to diagnose or to treat. Therefore, C-RIDL aims to emphasize the importance of the correct use of both Rıs and CDLs and to encourage laboratories to specify the appropriate information to clinicians as needed.
The aim of this talk is to present the current theory and practice of Rıs together with a detailed evaluation of the most recent multicenter studies, an assessment of the Rıs of the pediatric and geriatric groups, which is still regarded as a problem in this area, a detailed explanation of the advantages and disadvantages of the indirect approaches, future possibilities based on the comparison of direct and indirect methods for establishing reference intervals and a clarification of the confusion which arises from the use of CDLs.
Keywords: IFCC, C-RIDL, reference intervals, multicenter studies, EP28-A3c guideline, decision limits, indirect methods

Developing a roadmap for laboratory test utilization management program

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Utilization management has been a traditional approach to control costs in clinical laboratory services for several decades. Following utilization management, best practices results in the highest quality care at the lowest cost, supports Lean and Six Sigma initiatives, and saves significant time and money. In fact, appropriate utilization reduces patient risk and empowers organizations to provide the highest quality of care. While it is good to have an understanding of utilization management, IFCC Committee on Clinical Laboratory Management has recently conducted an international survey to investigate what does this mean for the laboratory leaders and examined the state of medical laboratory test utilization management and relevant practices which are country-specific from a laboratory leader perspective. The findings of this survey revealed that the recognition of test utilization management, current practices, and maturation of those programs are significantly diverse among countries. It is relatively well established in most developed nations. However, the findings have confirmed that the need to develop a roadmap and to construct essential strategies for managing laboratory test utilization is a common interest. With this regard, it is of importance to select the right management tool to implement an optimal laboratory test utilization. This presentation will address the following key points for implementing utilization management initiatives:
· Structure of effective communication
· Infrastructure to assist implementation
· Establishing a laboratory formulary
· Gatekeeping mechanisms
· Clinical decision support
· Benchmarking and management metrics
· Consultative and Interpretive services

Threat of chemical weapons in Syria conflict and its impact on Balkan Region along with the health and laboratory management system

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The Balkan countries have a special geostrategic importance as they enable the passage to the Mediterranean from North and cross region from Asia including Middle-East to Europa. Since this region has been one of the targets of various States and groups historically, this geographical area is still under the influence of terrorist attacks.
As one of such dangers, threat emerging from chemical warfare / terror agents has been one of the biggest challenges originated not only from military operations but also from terrorist attacks and natural disasters. The threat from chemical agents on this dynamic "hot" region is still a great concern necessitating an extensive collaboration between the Balkan countries.
From the beginning of the crisis starting at 2013, chemical weapons have been used over 160 times in Syria and at least more than 15000 Syrians have suffered from chemical exposure since the beginning of the conflict. Some intelligence analysis data reported that Syria had an important chemical weapon capability, which included blister agents, like sulfur mustard, and nerve agents, like sarin and Vx. The striking event of those which occurred in Syria was the attack of chemical weapons used on 21 August 2013 during the ongoing conflict between the parties in the Syrian Arab Republic also against civilians, including children, on a relatively large scale according to the UN Mission team reports. A high number of patients in a short period of time were showing clinical signs, like miosis, excessive secretions of rhinorrhea and salivation, shortness of breath and convulsions, were consistent with exposure to a nerve agent.
Since this threat has evolved as a great issue in both military and terrorist aspects in recent years, an effective medical preparedness against such weapons along with the effective health organization is a requirement. Chemical warfare/ terrorism is the intentional use of toxic chemicals such as nerve gases, vesicating agents, cyanide, toxic industrail chemicals, smokes, tear gases, cyanide and chemicals listed in the Chemical Weapons Convention and toxins to spread life-threatening diseases in order to incapacitate the population of an area. They are used for hostile purposes and planned to cause disease or death in human, animals or plants.
Under the pre-incident preparedness measures, A rapid and coordinated medical response should be based on main integrated areas of interest including training and research on preparedness and prevention,
detection and surveillance system,
diagnosis and characterization of the agents
and emergency management involving epidemiologic investigation, medical treatment and prophylaxis for affected people and decontamination measures.
Modern threats of biochemical terrorism lead to development of methods for true and fast detection of chemical weapons. Currently, there are many types of methods used in this field, from which chromatography techniques can be employed for more rapid identification of these agents than conventional laboratory analytical methods. However, analysis is often challenging because of the limited size, quality, and purity of the biological target for the verification of chemical in use.
Lack of coordination and preparedness at national, regional and international levels can have some dramatic consequences. Defense against chemical threat is such a complex issue that requires highly qualified experts from various organizations including medical units, procurement of protective measures and detection assets and being aware of the current treatment approaches accompanied with extensive training activities. This is why the medical management involves first responders' organisations (including health experts) in charge of the protection and mitigation of the effects of chemicals.
To enhance coordination and effective medical response against regional chemical threat, some proposals may include in fields of concern such as: sharing medical preparedness plans whic also contain emergency medical and public health issues, establishing specialized response teams and a laboratory response network, exchange information and publications which are not confidential, organizing scientific meetings to increase the awareness amongst medical personnel. A Balkan common understanding may play a vital role in coordinating and conducting these mentioned measures.
In this presentation, measures that needs to be taken against biochemical terrorism and concepts are to be reviewed from the Turkish medical perspective and potential items which are supposed to be roled in this event to be outlined. Through this presentation, a table top analytical laboratory exercise following a chemical terrorist attack will be simulated, and the response and coordination that needs to be developed against such an attack will be summarized within this network between the countries through the each national condiserations for coordination.

The new In Vitro Diagnostic Regulation 2017/746 and consequences for Laboratory Medicine

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The new In Vitro Diagnostic Regulation 2017/746 (IVDR) was published May 2017 and will fully replace Directive 98/79/EC (IVDD) per May 2022. The aim of the IVDR is to further establish a well-regulated and smoothly functioning single market for in vitro diagnostic tests (IVDs) within the EU that is better aligned with new developments and guidelines. The IVDR introduces scope enlargement and a risk-based classification of medical tests. In this era of genetic testing and companion diagnostics it was crucial to secure protection of patient safety and public health by setting high standards for safety and performance of IVDs. The IVDR brings along expanded involvement of notified bodies that have to assess the majority (~85%) of IVDs with respect to IVDR compliance (namely for class B, C and D tests). Other key changes are the requirements for evaluation and documentation of clinical evidence (i.e. scientific validity, analytical and clinical performance of tests); the introduction of a universal device identification code system (UDI); the set-up of an EudaMed database for the deposition by IVD-industry of information about IVDs and lot-specific data; and the establishment of ongoing post-market surveillance programs. IVD-manufacturers have to fulfil all these IVDR requirements in order to get CE-marking and market access under the new IVDR. These changes will translate to a need for additional well-trained staff, increased costs and a higher dependence on notified bodies (for class B, C and D tests) and expert panels (in case of class D tests) for IVD-manufacturers. The replacement also has major consequences for diagnostic labs that use Lab-Developed-Tests (LDTs).
Keywords: IVDR, IVDD, clinical evidence, notified bodies, expert panels

Galectin-3: from molecule to biomarker and back

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Galectin-3 (Gal-3) is today the most widely studied member of galactoside-binding lectin family (galectins), but during the first 25 years after it was firstly described in 1982 Gal-3 was mainly of interest of a few "glycobiology groups". During that period, a significant amount of knowledge about Gal-3 had been collected and Gal-3 was recognised as both a potential diagnostic biomarker and a therapeutic target. We learned that Gal-3 can be present intracellularly (in the nucleus and cytoplasm) or extracellularly (on the cell surface and in the extracellular space), found in a wide range of cells and tissues, but that its expression depends on cell/tissue type and maturity, whereas the cells in which it is the most abundant are macrophages, epithelial and endothelial cells. Besides, it was found that Gal-3 affects numerous biological processes (*e.g.* cell proliferation, differentiation, apoptosis, signalling, cell-cell and cell-matrix interactions, *etc.*) through the specific interactions with a variety of intracellular (protein-protein) and extracellular proteins (lectin-sugar). Furthermore, it was identified as an important player of many physiological and pathophysiological events such as inflammation, fibrosis, angiogenesis, tumorigenesis and metastasis, but still it was just a one of thousands molecules "with a great potential".
Although Gal-3 was previously recognised as a molecule related to the heart failure in animal model, the Copernican turn regarding the interest for Gal-3 occurred by the study of Kramer's group in 2008, conducted on end-stage heart failure (HF) patients. The study detected elevated Gal-3 plasma concentration in those patients immediately and 30 days after application of mechanical circulatory support. After this discovery, the interest of scientists and clinicians for Gal-3 tremendously increased, resulting in more than 600 papers (in PubMed) that referred "Gal-3 and heart" in the last 10 years. In 2013, the American College of

Cardiology Foundation and the American Heart Association recognized the value of Gal-3 testing and included it into the Guideline for the Management of Heart Failure, because it has been proven that Gal-3 could provide useful information for optimisation of HF patient care decisions. Namely, Gal-3, as a biomarker of myocardial fibrosis, is predictive of hospitalization and death and may provide incremental prognostic value over natriuretic peptide levels in patients with HF.
Gal-3 has also been proven as a useful diagnostic marker for the differentiation of benign and malignant thyroid nodules, whereas its value for the diagnosis/prognosis of other malignant and chronic diseases, *e.g.* diabetic nephropathy, is under intensive investigations.
Due to its important roles in different pathologies, Gal-3 has also been recognised as a potential therapeutic target. However, designing selective Gal-3 inhibitors is challenging because of the shared homology of the carbohydrate-recognition domains among not only galectins, but also other lectins. Yet, several Gal-3 agonists, either plan-based (GCS-100, GM-CT-01, GR-MD-02, modified citrus pectin) or synthetic (TD139) are in different phases of clinical trials as a potential drugs for different chronic diseases, *e.g.* NASH advanced fibrosis, chronic kidney disease, idiopathic pulmonary fibrosis, osteoarthritis, *etc*. as well as malignant diseases, *e.g.* chronic lymphocytic leukaemia, melanoma, colorectal cancer, metastatic melanoma, *etc*.
Our long-standing interest in Gal-3 has recently been directed on its involvement in the adaptation response of cardiovascular system (CVS) to recreational SCUBA diving, which represents a special form of physical activity, due to the body exposure to low temperature, hyperoxia and elevated pressure. Our studies of the effects of single dive and repeated dives on CVS, showed significant changes not only in Gal-3 plasma concentration, but also in the levels of other CVS biomarkers, such as hs-TnI, NT-proBNP, VEGF, endothelin-1 and myoglobin. Although transient, these changes suggest extensive activation of adaptation mechanisms, which in some aspects could possibly have a positive effect of SCUBA diving on CVS.

Serum non-coding RNA profiling as a promising diagnostic approach

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Serum non-coding RNAs (ncRNAs) have been identified as paracrine and endocrine messengers of different diseases. It has now been widely acknowledged that ncRNAs a new area in the field of biomarkers has emerged. ncRNAs are RNA molecules of different sizes that are transcribed as independent genes or as part of protein coding genes and are not translated, therefore they do not produce proteins. They have been classified according to their size and function and include micro RNAs (miRNAs), piwiRNAs (piRNAs), snoRNAs and long non coding RNAs (lncRNAs). These non coding RNAs are present in different cell compartments participating in multiple cell functions, but they have also been identified in biological fluids, also known as cell-free or circulating ncRNAs, where they can be detected in exosomes, bound on lipoproteins as well as free circulating molecules. The role of circulating ncRNAs is still under investigation but are believed to be paracrine or endocrine messengers to systematically deliver signals between cells and tissues. Extensive studies have implicated a family of ncRNAs, this of miRNAs in disease pathogenesis and their potential as diagnostic and prognostic biomarkers of diseases. Recent evidence have identified additional families of ncRNAs such as piRNAs or lncRNAs as potential diagnostic tools both in the serum and in tissues. Detecting ncRNAs in biological fluids has opened a new field in Clinical Chemistry utilizing them as biomarkers of diseases or prognostic markers for different pathological conditions. To date, individual ncRNAs or groups of ncRNAs are being used to facilitate disease diagnosis. Nevertheless, diversity between individuals and pathogenetic mechanisms limits their specificity for most conditions. As high throughput analyses are becoming wider used and more affordable, ncRNA profiling is emerging as a diagnostic and prognostic approach. Profiling utilizes next generation sequencing approaches and allows screening of all ncRNAs in biological fluids or cell extracts, thus providing a comprehensive view of the changes in any particular patient. Serum ncRNA profiling coupled with bioinformatics analyses that identify targets and functions associated with the target genes, provides evidence for a direct impact of the circulating ncRNAs on disease pathogenesis. A recent example published by our group has shown that ncRNA profiling identified miRNAs and piRNAs as biomarkers of male subfertility and associated those with hypogonadism. Additional examples in cancer patients have indicated that changes in serum ncRNA profiling reflects changes in cancer growth and may predict disease outcome. Thus, profiling of ncRNAs will provide a diagnostic tool that allows global understanding of changes occurring in diseases. Thus, ncRNA profiling coupled with proteomics analyses in patient samples is the foreseeable future in diagnostics.

Ethical issues in (pharmaco) genetics

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Apart from genetic testing for diagnostic purposes, application of genetics in human medicine encompasses genetic interventions and pharmacogenetical testing which are becoming more frequently utilized in clinical practice, as well as genetic studies employed in the process of research and drug development.
It's been widely known and accepted that application of a drug in equal dosing regimens for treatment of the same diagnosis in different patients, doesn't produce equal results regarding achievement of a therapeutic effect and/or occurrence of side effects. Investigating the genetic cause for interindividual variations in patients' drug response and toxicity, pharmacogenetics holds valuable prognostic and predictive value in tailoring the pharmacological treatment of various diseases accorcing to the principles of precision medicine.
But, just as any other medical testing, genetic analyses impose ethical risks which in this case are even more serious due to the following specific features of these tests and the obtained data: the "mutual" ownership of the genetic information by individuals from the same family, the lack of precise phenotype-genotype correlation and the influence of epigenetic and environmental factors on the phenotypic expression of genetic information, the balance between the right of an individual "to know" and the right "to not know" as well as the enormous potential for discrimination. The rapid advancement of high throughput technologies delivering a mass of detailed data on an individual's genome introduces a lot of advantages in scientific and clinical applications, but also threatens with a tremendous risk for misuse of these data in various settings.
The lecture discusses the fundamental ethical principles applicable to genetic analyses/studies including respect of the individual's autonomy and privacy and commitment to providing confidentiality, beneficence and justice. The informed consent as well as the levels of anonymization in genetic testing as measures to satisfy the above mentioned principles will be addressed. Special emphasis will be placed on the ethical issues regarding orphan and rescued drugs emerging in the pharmacogenetical testing within clinical studies in drug research and development.
Philosophers of science claim that science is morally neutral, it is actually the use and implementation of science that can have positive or negative impact. Hence, it is crucial to understand that achievement of our aim for humane application of (pharmaco)genetics can only be accomplished if technological and clinical advances in this field advance at a similar rate with the corresponding ethical considerations.

The relationship between adiposity parameters and hsC-reactive protein values in overweight and obese women

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Overweight/obesity has become an important health problem in developed countries and as a result of the rising epidemic of obesity, understanding body fat distribution and its clinical implications is critical to timely treatment. Adipose tissue is anatomically distributed in different proportions throughout the human body, but the percentage of adipose tissue is higher in women, the elderly and overweight individuals. Visceral adipose tissue is a hormonally active component of total body fat, which possesses unique biochemical characteristics that influence several normal and pathological processes in the human body. It has been distinctly linked to several pathological conditions including impaired glucose and lipid metabolism, insulin resistance, several malignancies, increased incidence of infections and non-infectious complications, and increased mortality

in hospital. Visceral obesity itself is an independent component of metabolic syndrome and the magnitude of obesity directly relates to the prognosis of this condition. It may be related to presence of low-grade inflammation in white adipose tissue. Precise mechanisms of chronic inflammation induction in obesity as well as the relation between obesity and inflammatory markers are yet to be explained. So far, the importance of high sensitive C-reactive protein (hs-CRP), as the most versatile inflammatory marker, is still in the spotlight. Hs-CRP outstands as independent risk factor, apparent from traditional risk factors such as increased total cholesterol, increased levels of glucose and homocysteine, hypertension, age, high body mass index (BMI), smoking and physical inactivity. As a hormonally active tissue, VAT releases different bioactive molecules and hormones, such as adiponectin, leptin, tumor necrosis factor, resistin and interleukin 6 (IL-6). Among these hormones, adiponectin is of particular significance owing to its protective antiangiogenic activity. In addition, in obese persons, white adipose tissue is infiltrated by macrophages with increased local production of proinflammatory mediators. These factors promote acute phase reaction and chronic inflammation in obese persons. However, some authors proposed the existence of a subgroup of obese persons who are metabolically normal (without increased risks of heart diseases, type 2 diabetes, hypertension, stroke, cancers, etc.). They hypothesize that in this subpopulation obesity seems to be uncomplicated and is characterized by early onset, hyper-plasticity of otherwise normal adipocytes, and peripheral type of fat distribution. The inflammation in these persons should be absent, and they supposed to have normal levels of inflammatory markers. The aim of this study was is to investigate the levels of inflammatory marker CRP and adiponectin and their relation to standard anthropometric parameters [body mass index (BMI) , waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR)], in population of apparently healthy overweight and obese females. This study enrolled 76 overweight (BMI between 25 and 29.9 kg/m2) and 45 obese (BMI ≥ 30 kg/m2) females, nonsmokers, aged 18–45 years, without any comorbidities, and with regular menstrual cycles. Standard anthropometric measurements were performed: body weight (BW), body height, WC and hip circumference and followed parameters were calculated: BMI [kg/m2], waist-to-hip ratio (WHR), waist-to-height ratio (WHtR). Quantitative determination of hs-CRP was determined using particle enhanced turbidimetric assay on the Cobas Integra 400 plus autoanalyser. The measuring range of hs-CRP was 0.1–20 mg/L, with a lower detection limit of 0.1 mg/L. Levels of the total adiponectin were measured by an ELISA competitive enzyme immunoassay for quantitative measurement of the human adiponectin, using commercially available kits (BV51001 Human Adiponectin). Average hs-CRP was 5.36 ± 2.43 mg/L, and significantly positively correlated to all investigated anthropometric parameters.

Statistical analysis showed the significant difference between the overweight and obese group for all investigated anthropometric parameters, except for the age as well as CRP values.

Average adiponectin was 9,88±4,4 and correlated both negatively and significantly with the waist circumference, BMI ($p<0.001$). The major characteristic of our results is significant difference observed between overweight and obese subjects in almost all important features. Besides the anthropometric differences which were expected (BW, BMI), in the overweight group we recorded significantly lower values of parameters that reflect the metabolic risk: WC, WHR, WHtR, as well as significantly lower values of inflammatory marker CRP. Our results confirmed that CRP is a valuable marker of metabolic risk in obese females, and BMI, although not so new, is still a reliable parameter of adiposity.

Photodynamic activity properties of novel BODIPY compound against colorectal cancer cell line

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Colorectal cancer (CRC) is the third most common cancer type and the second leading cause of cancer-related mortality worldwide in 2018 according to World Health Organization reports. Photodynamic therapy is a well-established clinical modality for treating various types of cancers. BODIPY compounds are promising molecules for diagnostic and therapy usage in cancer. In this study, photodynamic activity potential of water soluble novel BODIPY compound bearing pyridine group using different techniques were investigated. The photochemical and CT-DNA binding properties of water soluble novel BODIPY compound bearing pyridine groups (6a) were investigated absorption titration, competitive ethidium bromide and viscosity experiments. The DNA cleavage activities and topoisomerase I and II inhibition properties of compounds were investigated using pBR322 DNA on agarose gel electrophoresis. The cytotoxic and phototoxic effects of the compound were tested against human colorectal (HCT-116) cell line using MTT assay and flow cytometer. The singlet oxygen quantum yield of 6a was 0.21 in photochemical studies. The DNA binding experiments suggested that 6a interacted with DNA via non-covalent modes. 6a significantly cleaved pBR322 plasmid DNA forming singlet oxygen with light irradiation. The topoisomerases studies suggested that 6a inhibited enzymes in a concentration-dependent manner. In the cell culture studies, 6a had lower cytotoxic and higher phototoxic effects. In addition it induced apoptosis on HCT-116 cells. The results suggested that it was thought that 6a had a promising photosensitizer agent for CRC.

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Metabolomics and biomarkers in inborn errors of metabolism

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Inborn errors of metabolism are inherited disorders resulting from mutations affecting functional proteins such as an enzyme/transport/activator protein in the metabolic pathways or organelle function that cause an interruption of protein, fat, carbohydrate, sterol, nucleic acid, membrane, neurotransmitter etc. metabolisms. To date, over 1000 inborn errors of metabolism have been identified. Although they are individually rare disorders, the cumulative incidence is 1/1000 live births. Age of presentation can vary from infancy to adolescence with a wide clinical spectrum, the more severe forms appearing in early childhood accompanied by significant morbidity and mortality. Nowadays, treatment options including enzyme replacement, substrate reduction, cell and organ transplantation and gene therapies are available and early diagnosis is becoming important for early treatment. In recent years, with the development of high-througput technologies, metabolomic studies have advanced and new biomarkers have started to emerge for early diagnosis and treatment follow-up. Metabolomics is comprehensive analysis of metabolites (≤1500Da) in a biological specimen that can enable precision medicine at a number of levels, including the characterization of metabolic derangements and metabolic phenotypes that underlie disease, discovery of new therapeutic targets, and discovery of biomarkers that may be used to either diagnose disease or monitor activity of therapeutics. Structural and functional information on 247 metabolites associated with 147 inborn errors of metabolism and 202 metabolic pathways involved in various inborn errors of metabolism have been reported in the human metabolome database (HMDB). Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS)-based tecnologies are reference methods for extracting comprehensive and unbiased chemical information from complex mixtures of metabolites. Both targeted and untargeted mass spectrometry-based metabolomic approaches have been used to expand the range of disease-associate metabolites. In the targeted approach, specific metabolites are detected, quantified and compared to establish reference ranges. The untargeted approach consists of analysis of all detectable metabolites known and unknown in a single test performed on a biological sample to determine any perturbation of single or multiple metabolites and of related biochemical pathways. Among the first well-known targeted metabolomic for inborn errors of metabolism include acylcarnitine, amino acid and organic acid analyses in biological samples for screening of the disorders. Aminoacidopathies, organic acidurias and fatty acid oxidation disorders were investigated by using the targeted metabolomic analyses. Beside them, analyses of oxysterols (Cholestane-3β, 5α, 6β triol and 7-ketocholesterol) as biomarkers for Niemann-Pick Type C, and bile acids in the diagnosis of hereditary bile acid metabolism defects have been performed by targeted mass spectrometry-based analyses in Central Laboratory of Hacettepe University Hospitals. Classical bile acids, hydroxylated bile acids, 3β-hydroxy-Δ^5-bile acids, 3-oxo-Δ^4-bile acids, short-chain bile acids, long-chain

bile acids, differential bile acids can be screened in urine samples by LC-MS/MS. An increase in 3-oxo-Δ^4-bile acids in Δ^4-3-oxo-steroid 5β-reductase deficiency, an increase in 3β-monohydroxy-Δ^5 bile acids in oxysterol-7α-hydroxylase deficiency, an increase in bile acid alcohols in peroxisomal diseases can be observed. Decreasing in unusual bile acids can be observed during treatment follow-up. With targeted metabolic approaches using MS technology, biomarkers for different inborn errors of metabolism can be detected. Beside oxysterols, lyso-sphingomyelin-509 for Niemann-Pick Type C, glycosaminoglycans for types of Mucopolysaccharidoses, globotriaosylsphingosine (LysoGb3) for Fabry, Lyso-Gb1 for Gaucher are examples for biomarkers of some inborn errors of metabolism that can be detected by targeted metabolomic analyses. The best approach to metabolomic study of complex inborn errors of metabolism may be the combination of untargeted approach, that span the breadth of metabolome and perform pathways analysis, with targeted approach, that measures specific metabolites and establishes their reference intervals. The integration of genomic with metabolomic data will improve diagnosis and prognostication of inborn errors of metabolism.

Key words: Metabolomics, mass spectrometry, biomarkers, inborn errors of metabolism

Genetic Technologies in Inborn Errors of Metabolism

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Inborn errors of metabolism (IEM) are inherited single gene disorders caused by a deficiency of an enzyme, its cofactor or a transporter protein in synthesis or catabolic pathway of proteins, carbohydrates and fatty acids. Neurometabolic diseases, which are also considered as a subgroup of metabolic diseases, result in neuronal damage due to inability to synthesize essential biochemical substances, abnormal accumulation or formation of toxic metabolites. Although these disorders are individually rare, collectively they account for a significant portion of childhood genetic disorders.

The incidence of metabolic / neurometabolic diseases is generally reported as 1/4 000 - 1/5 000 in the worldwide. The rate of consanguineous marriages in Turkey is 21.4% resulting in a population with relatively high frequency of metabolic/neurometabolic disorders compared to other countries. Metabolic/neurometabolic disorders are considered rare diseases in other countries however these disorders are much more common in Turkey. IEM are quite heterogeneous and severe genetic diseases with a variety of overlapping or unspecific clinical phenotypes. Even though primary diagnosis of IEM is done by clinical suspicion and biochemical tests, genetic investigations play a significant role for appropriate patient treatment and genetic counselling as indispensable tool.

Since the sequencing of the first human genome in 2001, genomic technologies have made a huge impact across many fields such as medicine, computational and information technology, and healthcare. For many years there have been a variety of technologies and tools used in genome analysis. However, only in the past decade there has been rapid revolutionizing progress and improvement in high-throughput methods. These methods are ranging from traditional conventional laboratory genetic techniques of microscopic cytogenetics, fluorescence in situ hybridization (FISH), southern blotting, denaturing gel electrophoresis, single stranded conformation, restriction fragment length polymorphism (RFLP), mapping and genotyping studies using microsatellite markers and many other nonsequencing genetic laboratory methods to more complex systems, such as microarrays and next-generation sequencing. Developing these new methodologies allow rapid, high specificity and high-throughput and cost-effective analysis of a large number of samples from small amount of biological material. Also, utilization of advanced genetic technologies applications/analysis has led to the rapid and accurate diagnosis of the diseases and as a result several novel diseases have lately been defined.

IEM are generally inherited as autosomal recessive although dominant, mitochondrial and X-linked types of inheritance are also possible. The genetic defects include point mutations, deletions, insertions or chromosomal abnormalities that result in loss- or gain-of-function of mutant enzymes or proteins. Depending on the variant type and locus, there are numerous different genetic methods and tools for the variant detection. For example, due to its simplicity the most frequent method for the analysis of a large (>5 Mb) chromosomal aberration is karyotype analysis by using the GTG banding technique. Other molecular genetic methods, such as microarray-based comparative genomic hybridization (aCGH) or fluorescent *in situ* hybridization (FISH), could be applied for a more accurate analysis. Moreover, for detection particular variant another molecular genetic methods might be applicable, which include restriction enzyme assay of specific DNA sequence. Direct DNA sequencing method (Sanger sequencing) is accepted as the "gold standard" for the identification of known as well as unspecified variants such as point mutations, small deletions and duplications in the genomic DNA. Although the majority of the mutations accounting for IEM are point mutations, sometimes, large deletions and insertions and copy number changes can be causative. For this purpose, the most commonly used technologies are "multiplex ligation-dependent probe amplification (MLPA)" and "comparative genomic hybridization (CGH)." High-throughput single-nucleotide polymorphism (SNP) microarray is also produces genome-wide results that designed for genotyping a patient's DNA for genome-wide association studies (GWAS) and co-segregation studies to determine linkage between a disease locus and a chromosomal region These results are very useful in complementing the next-generation sequencing results. Furthermore, SNP array platforms may now also include a large number of probes specifically for the detection of copy number variants. SNP arrays and aCGH arrays also provide accurate tools for detecting small deletions and duplications.

In recent years "next-generation sequencing technologies" have enabled investigation of hundreds and thousand of targeted genes, even the whole exome and whole genome, at single base pair resolution. Large deletions and insertions can also be detected by this technology. The use of whole exome sequencing (WES) has facilitated the identification of the many novel genes responsible for metabolic/neurometabolic diseases. In terms of rare genetic diseases, the identification of the genetic basis of disease is reached in 20 to 40% of patients using WES. The most important and crucial part of exome and genome sequencing, is to find the disease-causing mutation among the thousands of variations. The structural or functional effect of the variation on protein or enzyme determines whether the nucleotide changes are a mutation that causes disease pathology. Base changes that result in amino acid changes (missense mutations) are evaluated by software programs including PolyPhen-2, SIFT, and Mutation Taster that rate the pathogenicity of the amino acid change. These programs estimates of how tightly the base is conserved over evolution, whether the amino acid changes charge, size, or conformation, and, sometimes, where in the protein the amino acid change reside. Functional studies may be required to demonstrate the pathogenic effect of a mutations. Genome databases, disease databases, mutation databases can be used to investigate clinical phenotypes caused by different mutations.

Depending on the location and type of mutations, their effects on protein and clinical findings in patients may vary widely. Mutations that cause clinical phenotype are now being investigated by comprehensive methods including collectively evaluating all changes at the genomic level, searching for modifying genes, epigenetic changes and genome-wide bioinformatic analysis methods. Molecular diagnostic methods are of increasing importance in many medical applications such as elucidating the pathophysiology of the disease, identifying patients and carriers and providing genetic counseling services, identifying asymptomatic individuals, differential diagnosis of atypical patients, and developing effective treatment and follow-up for patients.

CONGRESS COURSE ABSTRACTS

EFLM Course: How to write a good scientific and professional article?

Sverre Sandberg, Noklus, Norway - Elvar Theodorsson, Ike/Klinisk Kemi, Sweden

Introduction
Scientific manuscripts can be written in solitude, but it is commonly more productive and more likely to result in better quality if several persons collaborate. The present course builds on this premise and on the understanding that mentored learning through an intensive work of the participants themselves is the most effective method of learning.
The present 48 hours EFLM course in the writing of scientific manuscripts rests on four pillars:
The task of turning a scientific study with resulting data that the participants get from the outset into a manuscript written in English as when intended for publication in an international scientific journal
The participants work closely together in groups of 5-8 persons chosen by the mentors. The group work is performed in the participants own mother tongue whereas the communication of the group with the mentors and in the written parts of manuscript will be in English.
The individual participants are expected to take and maintain the initiative during the entire course in creating and maintaining a fruitful working relation in the groups and in utilizing their own knowledge and skills and those of the two mentors optimally for finishing the task of the group in a fruitful and timely manner.
The two mentors will have brief introductory lectures and subsequently rotate amongst the groups for advice on request of the members of the groups and for making short (about 20 min) presentations.
The tasks of the participants
Maintaining an open mind regarding the knowledge and skills of all other members of the group
Contributing all the relevant knowledge and skills of the participants towards the common goals of the group
Introductory lectures of about 20 minutes each
Day 1 The study and data that makeup the data for the current manuscript
• The Material and methods part of the manuscript
• The Results part of the manuscript
• The Introduction part of the manuscript
Day 2 The Discussion part of the manuscript
• The Title, index words and Abstract parts of the manuscrip

miRNA isolation and expression training program

Aylin Sepici Dincel, Gazi University, Turkey - Oytun Portakal, Hacettepe University, Turkey

miRNAs regulate various metabolic and cellular pathways that specifically control cell proliferation, migration, differentiation and signal transduction by negatively affecting the expression of target mRNAs. Numerous microRNAs have been identified that play a role in the transcription and translation of tumor suppressor genes or oncogenes in many solid tumors and hematologic malignancies. The relationship between microRNA expression levels and metastasis and survival in cancer patients was investigated. Since microRNAs have tissue-specific expression profiles and effective and reliable methods of expression quantification can be performed, microRNA-based markers are important in cancer diagnosis, prognosis and follow-up of treatment. In addition, microRNAs are involved in the regulation of radiotherapy and chemotherapy responses of tissues.
Objective
To gain knowledge and ability to detect and evaluate the amount of miRNA expression from different samples. The theoretical part of the course will provide information on miRNA isolation methods and details, methods for determining the amount of miRNA, amplification methods and how to normalize them. For the isolation of miRNA, the methods of isolation from classical tissue or cell culture as well as direct isolation from plasma will be discussed and their effectiveness will be examined. Sources of errors and strategies to avoid mistakes will be explained. In the application part, suitable samples prepared for miRNA isolation will be used and individual study will be provided. The quantification and expression of miRNAs obtained will be discussed with visual materials prepared by real-time PCR method. Information will be given about normalization. Clinical evaluation of identified up-regulated and down-regulated miRNA profiles will be performed.
Learning Objective
To be able to create a different view of these molecules with the recent developments in the field of research of miRNAs that attract attention with biomarker studies.
At the end of this course participants will:
1. explain and apply miRNA isolation methods from plasma and different tissues
2. be able to quantify miRNA
3. identify miRNA expression profiles
4. apply normalization, basic clinical evaluation issues
Target group
Researchers and postgraduate students and specialists in biochemistry.
Necessary knowledge and skills
Molecular genomic techniques should have a basic level of knowledge.

Applying Six Sigma to analytical performance in the medical laboratories

Hassan Bayat, Iran

Introduction
14:45-15:15
The concepts of Sigma Metric
The history of the development of this metric
Definitions of Acceptable Product, Acceptable Performance, the meaning of Defects per Million Opportunities (DPMO)
Two Approaches to Calculating Sigma-metric
15:15-16:00
The counting approach vs. statistical approach to determine defect rate
Statistical approach is appropriate for estimating error rate of analytical phase
Sigma-metric in Evaluation of Analytical Performance
16:00—16:45
The equation to determine the analytical Sigma-metric
The components necessary to calculate Sigma-metric: TEa, bias and imprecision
Sigma-metric in QC Planning
16:45-17:45
The relation between Sigma and patient risk
Characteristics of statistical quality control
Adjusting QC based on Sigma: QC rules, number of QC assays per event, and QC frequency
Q & A
17:45-18:15
The audience will present their comments and questions

Mass Spectrometer use in clinical laboratory practice (Basic Course)

Prof. Dr. Ali Ünlü, Selçuk University, Konya
Prof. Dr. Muhittin Serdar, Acıbadem University, İstanbul
Doç. Dr. Sedat Abuşoğlu, Selçuk University, Konya

AIM: Mass spectrometric techniques are increasingly used in clinical laboratory. The aim of this course is to reach a sufficient level of knowledge about working principles, procedures, aspects to be considered and application areas of mass spectrometry.
COURSE OUTLINE:
Liquid chromatography principles,
Mass Spectrometer (MS) techniques
Setting up a clinical mass spectrometer lab, calibrator and reagent selection in MS methods, quantitative result evaluation.
MS applications in clinical laboratories, comparison with immunassays.
Method validation and verification process in MS
Discussion
PARTICIPANT PORTFOLIO:
Medical biochemistry specialists, PhD, research assistants, graduate students, device users.

Mass spectrometer use in clinical laboratory practice (Advance Course)

Prof. Dr. Ali Ünlü, Selçuk University, Konya
Prof. Dr. Muhittin Serdar, Acıbadem University, İstanbul
Doç. Dr. Sedat Abuşoğlu, Selçuk University, Konya

AIM: Advanced mass spectrometer training course for partially experienced specialists who have mass spectrometer in their laboratory and familiar with mass spectrometer use.
COURSE OUTLINE:
“In house “method development in mass spectrometer laboratories and verification of commercial kits (CLSI 62-A)
Maintenance of mass spectrometry and troubleshooting, maintenance of quality in mass spectrometry analysis
Use of MS in metabolism laboratories
Method selection in analytical toxicology
Mass spectrometry use in endocrine laboratories
Discussion
PARTICIPANT PORTFOLIO:
Medical biochemistry specialists, PhD, research assistants, graduate students, device users.

INDUSTRY SPONSORED SYMPOSIUM ABSTRACTS

INDUSTRY SPONSORED SYMPOSIUM 1
BECKMAN COULTER

The role of autoverification in postanalitic process improvement

Özlem Gülbahar, Turkey

Today, there is an enhancing workload in clinical biochemistry laboratories because of the increase in test volume and variety. However, expectations of clinicians are increasing from the laboratory, including faster results. One of the solutions to cope with this situation is to use auto-verification (CDSS: Clinical Decision Support System). By CDSS, both the probability of human error and the turnaround time (TAT) will be shortened. More importantly, more time can be devoted to reviewing complex test results that require expert interpretation. Thus, it may be possible to develop rational laboratory use applications such as reflective testing and consultation. However, all these benefits depend on the creation of a suitable and adequate verification algorithm. Basically, an algorithm should include evaluation parameters for preanalytical phase (serum index, etc.), analytical phase (flags etc.) and postanalytical phase (critical value, delta check, etc.). In addition, validation studies must be completed, and reliability should be tested before use. The relevant CLSI guidelines (AUTO 10A and AUTO15) can be used for CDSS use. The use of CDDS, which is becoming more widespread in Europe and the USA, has started to be applied not mandatory in our country and its use is controlled by the health authority.

INDUSTRY SPONSORED SYMPOSIUM 2
SNIBE

The clinical performance of Maglumi AMH, 17-OH progesterone and B2 microglobulin

Pinar Eker, Turkey

After seeing the evaluation of post-analytical phase as laboratory specialists by taking the opinions about outcomes of patients regarding the available results of 17 OH progesterone, AMH, β2 microglobulin and free testosterone(it will be called Maglumi panel in the next), the survey is made which is for clinicians about the clinical usefulness of our results especially for diagnosis and monitoring of concerning diseases .
With the help of LIS data, the most frequent test requesting clinicians and the clinicians who requested the Maglumi panel tests have been chosen. In this case, The questionnaire was prepared and directed to each clinician by an e-survey in an electronic environment including the purpose where the results were graphically evaluated.
In terms of its contribution to clinical monitoring, positive feedback is received from most clinicians. Among all feedback, no less than 92% positive feedback is given expect that free testosterone with 84% positive feedback on monitoring, however, 92% positive feedback is given on its diagnostics use.
In conclusion, total testing process may be never ending circle. Communicating with clinician is another extra step we need for patient safety.

INDUSTRY SPONSORED SYMPOSIUM 3
ROCHE

Automation solutions in laboratories

Cigdem Karakukcu
Turkish Ministry of Health, Kayseri City Hospital, Biochemistry Laboratory

XXX. National Congress of the Turkish Biochemical Society TBS 2019 and XXVII. Balkan Clinical LaboratoryFederation Meeting BCLF 2019 have been hold together on October 27-31, 2019, in Antalya, Turkey. During the congress, Mrs. Karakukcu made a satellite symposium about the benefits of the workflow automation systems and she shared her experience during the installation and re-structure processes of the Biochemistry Laboratory located in the Central Laboratory, in the Kayseri City Hospital, one of the biggest hospitals of Turkey, with a 1607 bed capacity.
The start and installation of the biochemistry lab was on May 2018 without an automation system. During that time, there was 3,400 samples and 33,350 clinical chemistry and immunoassay tests daily and rate of Immunoassay testing was 19.3% of total. Under these conditions, the average Turn Araound Time (TAT) was 370 minutes. The whole system and company was changed on October 2018. System workflow was rearranged.
After the change in system on October 2018 (just only the change of analyzers and workflow, the automation system was not installed yet), daily run test number was 36,070. TAT decreased to an average of 114 minutes on January 2019. In this period, Immunoassay testing rate was 22.4%.
After the automation system was installed in January 2019, the number of laboratory tests reached 40,028 in September 2019. The average TAT was 135 minutes, although the immunoassay test rate reached 25%.
The addition of an automation system increases the control of the laboratory. It also has the effect of improving the workflow and eliminating errors in such high-volume laboratories. The contributions of automation to the laboratory can be summarized under 3 main pillars: Quality, Flexibility, Short and Predictible TAT.
Sample quality is checked in the first stage with a high resolution camera. In case the samples are lipemic, icteric or hemolytic, scenarios are defined and the journey of the sample in the laboratory is determined.
Sample loading to the system can be done with a bulk loader module or via a separate input module. The centrifuge module can change the rotational speeds according to the test contained in the samples. For example, it can be defined as 9 minutes rotation time for hepatitis tests and 7 minutes for other tests.
Since the carriers which carrying the samples in the automation system are also the carriers of the analyzers themselves, the samples are not transferred to another carrier while entering the analyzers. Thus, there is no time loss during the transfer of the samples.
In the automation system, a post analytical device with a capacity of 27,000 samples is used to archive and store samples at 4-8°C. Samples that complete the storage period defined as 3 days are sent to waste automatically by the system.
Due to the structure of the City Hospitals, the systems are expected to be quite suitable for expansion. The Cobas ® Connection Modules (CCM) system meets this expectation quite well. It is possible to replace the analyzers with faster models and to add new pre-analytical equipments to the system. In addition, the system can be extended to connect not only biochemistry analyzers but also hemogram and coagulation analyzers.

INDUSTRY SPONSORED SYMPOSIUM 4
MINDRAY

Clinical utility of Reticulocyte Hemoglobin and Hypochromic erythrocytes reported by Mindray BC6800 Plus hematology analyzer in the study of erythropoiesis

Eloísa Urrechaga, Senior Consultant for Clinical Laboratory

The hemogram is one of the more required tests by the clinicians, the analysis nowadays is totally automated, and the correct interpretation of the results requires joining the knowledge about the characteristics of the equipment and the clinical meaning of the results. The suppliers contribute innovations, providing new parameters that can help the clinicians to make a diagnosis in a fast, cheap, and useful manner.
Flow cytometry provides information about individual cell characteristics. This is in contrast to previous measurements of MCV, MCH, and MCHC which only calculate mean values for the total red cell population. Modern counters can provide information about the reticulocyte counts but also about the characteristics of these cells (size or Hb content), related to the quality of the erythropoiesis, giving information of the current erythropoietic activity of the bone marrow.
Mindray (Shenzhen, China) has recently launched a new analyzer 6800 Plus, which incorporate the RBC extended parameters, RBC subsets and the reticulocyte Hb content.
These parameters expand information at a cellular level:
(1) Provide information of the Hb on individual red cells, erythrocytes and reticulocytes as well
(2) Can aid in monitoring changes in Hb synthes

ORAL PRESENTATIONS ABSTRACTS

O-001
Thymoquinone and Sorafenib as a therapeutic combination in liver cancer: In vitro and in vivo

Eray Metin Guler, Abdurrrahim Kocyigit
Bezmialem Vakif University, School of Medicine, Department of Medical Biochemistry, Turkey

OBJECTIVES:Hepatocellular carcinoma (HSC) is the most common primary malignant tumor of the liver originating from hepatocytes.The aim of this study is to investigate the antitumor effects of chemotherapeutic agent sorafenib and N.sativa's active substance thymoquinone against in vivo and in vitro hepatocellular carcinoma.
MATERIALS and METHODS:Cytotoxicity, genotoxicity, apoptosis, intracellular ROS, intracellular glutathione and mitochondrial membrane potential were measured in Luc-transfected hepatocellular carcinoma cells. Cells were given to nude mices by zenograft method. TQ, sorafenib, and combined therapy reduced tumor size after the 4 week treatment. Tumor size were measured with an IVIS imaging device and caliper.
RESULTS:In vitro dose-dependent thymoquinone and sorafenib have cytotoxic genotoxic, apoptotic and ROS-producing effects, both individually and in combination. In vivo study, combine therapy was found to be more effective than mono therapies in vivo hepatocellular carcinoma, which was formed by the xenographic method. While tumor size, inflammation, oxidative stress decreased, While tumor size, inflammation, oxidative stress decreased, antioxidantive markers and thiol levels increased.
CONCLUSIONS:Our results showed that, thymoquinone has anticancer properties in vivo and in vitro. It has been shown to be more effective at lower doses when used with routine therapy.
Keywords: Thymoquinone, Sorafenib, hepatocellular carcinoma, IVIS

O-002
Investigation of type I collagen and MMP-2 changes in mandibular bone tissue in natural development

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OBJECTIVES:Mandibular bone, which is a part of the masticatory system, changes in histology and molecular structure based on the age and gender of an individual. The masticatory system develops with age and it affects all oral and temporomandibular joint disorders. In this study, we have aimed to examine the effects that aging has on the changes on type I collagen, which exists in the bone tissue and provides its matrix and its durability, and matrixmetalloproteinase-2 (MMP-2).
MATERIALS and METHODS:14 Balb / C species white mice were used in the study. Animals were divided into two groups of seven, based on whether they are young or old. Mandibular bone tissue homogenate was prepared for biochemical analyses and mandibular bone tissue was obtained for histological evaluations. After routine histological follow-up, the tissues were embedded in paraffin. 4-5 µm thick sections were taken from paraffin-embedded tissues and hematoxylin-eosin, Type I collagen and MMP-2 immunohistochemical stainings were performed.
RESULTS:Ca+2, ALP ve calcitonin levels were decreased in the aging-based bone tissue homojenate analyses that were performed and TNF-α and PTH levels were significantly increased. Type I collagen and MMP-2 immunoreactivity in elderly mice showed a significant decrease in comparison to young mice.
CONCLUSIONS:As a result, aging causes a decrease in the amount of bone formed in the bone reconstruction cycle due to the decrease in the osteoblast support and the increases osteoclastic activity
Keywords: Type I collagen, MMP-2, Calcium, Calcitonin, TNF-α

O-003
Induction of APAF-1 and TRAIL by bilberry tea in HCT-116 colon cancer cell line

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[3]Department of Food Engineering, Ege University Faculty of Engineering, İzmir, Turkey

OBJECTIVES:In this study, it was aimed to determine the effect of bilberry tea samples on the markers of the intrinsic and extrinsic pathways of apoptosis in the HCT-116 colon cancer cell.
MATERIALS and METHODS:Bilberry tea in different infusions and boiling (1 min, 3 min, 5 min, 7 min, 10 min) were prepared and phenolic levels were determined by LC MS / MS technique. The highest phenolic content was determined in tea samples of seedless fruits for 5 min boiling, so this product was chosen for in vitro study. Cytotoxicity and viability tests were performed by adding WST-8 solution. Intrinsic and extrinsic pathways of Apoptosis were assessed by determining the TRAIL, APAF-1,Cytochrome-c, Caspase -3,-8,-9 levels in HCT-116 colon cancer cell line.
RESULTS:Cytotoxicity studies in cell culture were conducted using 50-10 µg/ml of bilberry tea samples which was prepared at a concentration of 5 g/10 ml. The levels of APAF-1, TRAIL and Cytochrome-c were significantly higher in bilberry added cell culture than the control cells. Other markers (caspase -3, -8, -9 levels) did not show any significant change compared to control cells
CONCLUSIONS:It is concluded that bilberries induced TRAIL, APAF-1, Cytochrome-c and consequently induced both intrinsic and extrinsic pathways of apoptosis.
Keywords: APAF-1, TRAIL, Bilbery, HCT-116, Colon cancer

O-004
Induction of apoptosis and cell cycle arrest by pomegranate extract and tangeretin in the rat mammary carcinogenesis

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[3]Department of Medical Pathology, Faculty of Medicine, Firat University, Elazıg, Turkey

OBJECTIVES:The present study investigated the potential chemoprevention effects of Pomegranate extract (P) and Tangeretin (T), both alone and in combination, on the apoptosis and cell cycle in 7,12-dimethylbenz [a] anthracene (DMBA)-induced rat mammary carcinogenesis.
MATERIALS and METHODS:Sprague Dawley female rats (n=56) were randomly divided into 8 groups. Group I was control, Group II, III and IV were treated with P, T and P+T respectively. Group V was DMBA-induced (a single dose of 60 mg/kg body) weight breast cancer-bearing rats. Group VI, VII, VIII were designed as the chemopreventive treatment groups and were composed of D+P, D+T, D+P+T groups, respectively. The presence of the breast tumour tissue was demonstrated with histopathological examinations. In the breast tissue samples, the expressions leves of p53, Bax, Bcl-2 and cyclin D1 proteins acting on apoptosis and cell cycle were performed by western blot analysis.
RESULTS:According to histopathological evaluations, it was determined that most (90%) of the tumours created were invasive ductal carcinoma. While p53 and Bax expressions of pro-apoptotic markers significantly decreased in the DMBA group compared to the control group, it was observed that Bcl-2 and cyclin D1 expressions significantly increased. It was observed that p53 and Bax expressions significantly increased in both D+P and D+P+T groups compared to the DMBA group. Cyclin D1 expressions were determined to significantly decrease only in the D+T group.
CONCLUSIONS:Our study results have shown that the combined administration of Pomegranate extract and Tangeretin may be more beneficial in preventing breast cancer.
Keywords: Breast cancer, Apoptosis, Cell cycle arrest, Pomegranate extract, Tangeretin

O-005

Preparation of magnetic nanoparticle coated glutaraldehyde to reduce toxic effects of idarubicin and its effect on HL60 cell line

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OBJECTIVES:Anthracyclines (doxorubicin, daunorubicin and idarubicin) are very effective chemotherapeutic drugs to treat many cancers; however, they cannot distinguish between healthy cells and cancer cells and cause serious side effects and systemic toxicity. Furthermore, one of the major problems is that the drugs which are being used cannot be used efficiently because of their low half-life time and low stability. In recent years, studies have focused on magnetic nanoparticles (MNP) which are capable of carrying drugs to overcome these shortcomings. The aim of this study was to immobilize idarubicin (IDA) to glutaraldehyde-coated MNPs, to prepare a drug with high stability and low toxicity levels.
MATERIALS and METHODS:MNPs were prepared and coated with glutaraldehyde, IDA was immobilized and its activity in HL-60 cell line was examined. All of the materials were characterized by various measurements, including XRD,TEM, SEM and UV-Vis. Idarubicin loaded MNPs were administered to HL60 cell line at different doses, and MTT and ATP cell viability analyzes were performed and compared to free idarubicin.
RESULTS:The in-vitro cytotoxicity results showed that the IC50 value of IDA-MNPs was 13-folds lower than that of free IDA solution in HL60 cell line (IC50: 0,029µM for IDA-MNPs and 0,396µM for free IDA). In addition, analyzes showed that idarubicin was bound to MNP system by 54%.
CONCLUSIONS:The results of this study showed that MNP-induced idarubicin is effective in eliminating cancer cells even at doses 13 times lower. So these results show promising effects in cancer treatment.
This study was supported by TUBITAK BIDEB 2218.
Keywords: Magnetic nanoparticles, HL60 cell line, glutaraldehyde, idarubicin

O-006

The effects of overexpression of acetylcholinesterase on amyloid precursor protein and β-secretase-1 levels in Hs766T cells

Kevser Biberoglu, Seda Onder, Ozden Tacal
Department of Biochemistry, School of Pharmacy, Hacettepe University, Ankara, Turkey

OBJECTIVES:Acetylcholinesterase (AChE) plays a key role in catalytic hydrolysis of acetylcholine. It has known that acetylcholine can cause angiogenesis, migration and proliferation of cancer cells via activating the nicotinic acetylcholine receptor. Intensive research has indicated that AChE-R (readthrough isoform) is involved in proliferation, whereas AChE-T (tailed isoform) plays a role in apoptosis. A recent study has shown that AChE-T has potent anti-tumor effects and causes apoptosis of gastric cancer cells in-vitro and in-vivo. With the discovery of non-classical functions of AChE on cancer cells, the proteins that interact with AChE have become remarkable. Inhibiting the expression of amyloid precursor protein (APP) or β-secretase-1 (BACE1) is one of the therapeutic strategies due to their positive effects on cancer cell proliferation. In this study, we wonder whether AChE-T has any effects on APP and BACE1 expression in Hs766T pancreatic cancer cells.
MATERIALS and METHODS:Hs766T cells were transiently transfected with pGS-AChE-T plasmid, using lipofectamine-2000. To check transfection efficiency, AChE activity was assayed spectrophotometrically. After 48 hours of transfection, the levels of APP and BACE1 in cell lysates were analyzed using Western Blot.
RESULTS:We observed a significant decrease in both APP and BACE1 levels in transfected cells compared to vehicle-treated cells. Mature and immature APP levels were reduced by 60% and 68%, respectively whereas mature and immature BACE1 levels were reduced by 30% and 71%, respectively.
CONCLUSIONS: AChE-T reduces the levels of BACE1 and APP in Hs766T cells therefore it may show anti-cancer effects.
Supported by a grant from Hacettepe University Scientific Research Projects Coordination Unit (HUBAB, TSA-2017-13929)
Keywords: acetylcholinesterase, β-secretase-1, amyloid precursor protein, pancreas cancer

O-007

Genome-wide CRISPR-Cas9 screening for identification of cancer essential genes in malignant pleural mesothelioma

Ece Cakiroglu, Ozlem Silan Coskun, Serif Senturk
Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkey; Izmir Biomedicine and Genome Center, Izmir, Turkey

OBJECTIVES:Malignant pleural mesothelioma (MPM), which accounts for 80-90% of all mesothelioma cases, is a rare cancer with an increasing incidence and low survival rates. Existing treatment options are limited to chemotherapy with a low success rate. Therefore, novel targeted therapies are needed. In this study, we applied genome-wide negative selection CRISPR-Cas9 screening to identify cancer cell essential genes in MPM cell lines.
MATERIALS and METHODS:To obtain stable Cas9 expression we transduced 3 different MPM cancer cell lines and 1 normal epithelial cell line with lentiCas9-EGFP vector. FACS was performed to obtain and select clonal sublines with highest Cas9 expression. Competition assay and T7E1 assay were performed for functional characterization of selected clones. Brunello gRNA library was amplified and lentiviral particles were produced. Selected clones were transduced with Brunello gRNA library at MOI~0.3-0.5 and selected with puromycin and were cultured for 14 doublings.
RESULTS:We obtained clonal sublines showing permanent Cas9 nuclease expression. Selected clones with highest Cas9 expression were functionally characterized and were screened by transducing with whole genome Brunello gRNA library. Fold coverage of >400x was achieved following transduction.
CONCLUSIONS:Although whole genome CRISPR-Cas9 screening has some challenges due to the usage of high volume of cell cultures. While the risk of skewing of the composition of the final recovered DNA is high, CRISPR-Cas9 screening is still a powerful tool for obtaining essential genes and druggable targets in cancer.
This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) (Project number: 117Z227)
Keywords: CRISPR-Cas9 screening, brunello library, malignant pleural mesothelioma

O-008

The importance of serum hyaluronidase measurement in discrimination of patients with prostate cancer and benign prostatic hyperplasia

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[1]Health Sciences University Ankara Health Application and Research Center, Department of Medical Biochemistry, Ankara
[2]Health Sciences University Ankara Health Application and Research Center, Department of Urology, Ankara

OBJECTIVES:The aim of this study was to investigate the ability of serum HYAL activity and mass concentration to distinguish prostate cancer (PC) from benign conditions.
MATERIALS and METHODS:Our study included age-matched 37 newly diagnosed PK, 72 benign prostatic hyperplasia (BPH), 53 chronic prostatitis (CrP) patients according to biopsy results and 49 control patients. Other cancers, liver disease, rheumatologic diseases, collagen tissue disease and dermatological disorders that could increase serum HYAL levels were excluded. Morgan-Elson colorimetric method was used to measure serum HYAL activity (sHYALa). Serum HYAL concentration (sHYALc) was determined by an ELISA method. Biopsy results were used for evaluation of clinical performance.
RESULTS:sHYALa, sHYALc and total PSA levels were found to be significantly higher in PK patients compared to control and benign patients ($p<0.05$). In all groups, there was a relatively weak positive correlation between sHYALa and PSA (rho=0.405, $p<0.05$, n=141); sHYALc and PSA (rho=0.344, $p<0.05$, n=88). sHYALa and sHYALc was found to be significantly higher in PK patients with PSA values in gray zone (4-10 µg/L) compared to other benign patient groups ($p<0.05$). In ROC analysis, AUC for sHYALa, sHYALc and PSA were 0.866;

0.826 and 0.813, respectively. Sensitivity and specificity were found for sHYALa, sHYALc and PSA as 88%, 71%; %82, %89 and 79%, 71%, respectively.
CONCLUSIONS:Combining sHYALa or sHYALc with PSA, physical examination and ultrasonography data may be useful in the evaluation of PK patients.
Keywords: Hyaluronidase, Prostate, Cancer

O-009
Antioxidant and anti-denaturation activities of asparagus horridus grows in North Cyprus

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OBJECTIVES: Asparagus horridus is an edible plant known as "Ayrelli" in North Cyprus. There is a huge information gap in literature about this plant. The purpose of the research was to determine the antioxidant and anti-denaturation activities of the Asparagus horridus.
MATERIALS and METHODS:In this study, soxhlet extraction was used to obtain the extract from air-dried Asparagus horridus plant. We conducted a 1,1-diphenyl-2-picrylhydrazyl (DPPH), total flavonoid content (TFC),Ferric reducing activity and total phenolic content (TPC) tests to determine the antioxidant activity with using standard methods. Protein degredation assay was performed to determine the anti-denaturation activity of Asparagus horridus extract.
RESULTS:The DPPH test of Asparagus horridus methanol extract showed an increase of DPPH scavenging activity from 27.71 % ($p<0.0001$) to 49.69 % ($p<0.0001$) with the extract dose from 15 to 25 mg/ml.Total Phenolic Content of the extract was determined as 140.68 ($p<0.01$) to 167.61 ($p<0.01$) mg/µg equivalent of gallic acid with the extract dose from 15 to 25 mg/ml. Beside that Total Flavonoid Content was obtained as 119.72 ($p<0.00001$) to 273.5 ($p<0.00001$) mg/µg equivalent of quercetin with the extract dose from 10 to 25 mg/ml.Ferric reducing activity varied from 0.36 ($p<0.001$) to 1.27 ($p<0.0001$) mg/µg equivalent of $FeSO_4$ with the extract dose from 10 to 25 mg/ml. When anti-denaturation activity of Asparagus horridus extract was checked, is was found that the extract exhibited the highest inhibitory activity at 25 mg/ml as % 29.41±0.34.
CONCLUSIONS:Consequently, these results showed that the methanol extract of Asparagus horridus plant grows North Cyprus has important antioxidant and anti-denaturation potential.
Keywords: Asparagus horridus, North Cyprus, antioxidant, anti denaturation

O-010
CA125 test request ratio in male patients

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OBJECTIVES:Cancer Antigen 125 (CA125) test is one of the most commonly studied tumor markers in clinical biochemistry laboratories. Low sensitivity and specificity of CA125 restrict the clinical use of it. In addition to ovarian cancer, it may increase in tumor-related diseases of serous membranes and a number of benign conditions.CA125 test can be ordered by all clinicians in our hospital. We examined CA125 test orders in detail in the 6-month period and detected unnecessary test orders.
MATERIALS and METHODS:CA125 test was performed by chemiluminescence method on Advia Centaur XPT(Siemens) analyzer in our laboratory. CA125 which were analyzed between January – July 2019 period were examined from laboratory information system (ALIS, Ventura)(Reference Range: 0 - 35 U/mL).
RESULTS:In the 6-month period, 5,635 CA125 tests were performed which 1,356 of them belong to male patients(24%). In those patients, 144(10%) results of the ordered CA125 were found above the reference range(min:36, Max:4,463, median:87.5). Oncology(35%) and Internal Medicine(30%) clinics were having most common orders of CA125.
CONCLUSIONS:Unnecessary tests increase the laboratory workload and high costs. The use of tumor markers for screening in patients having no symptoms is one of the most common reason of the unnecessary test ordering. The main usage of CA125 test is non-mucinous ovarian carcinoma. Therefore, except in exceptional cases, it should not recommended for male patients.Department-based and/or gender-based test ordering restrictions through the hospital information system may prevent unnecessary test ordering, such as the order of CA125 for male patients. In addition a pop-up message can be created during the clinician orderings.
Keywords: CA125, unnecessary test request, tumor markers

O-011
Evaluation of tumor marker tests in a hospital setting

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OBJECTIVES:Early diagnosis and treatment of oncological diseases is extremely important. In this study, we aimed to evaluate tumor marker requests of our hospital and investigate the presence of improper use.
MATERIALS and METHODS:Evaluation of the tumor markers (CEA, CA 15-3, CA 19-9 and CA 125) performed by the biochemistry laboratory of Tokat Gaziosmanpasa Research and Application Hospital between 01.01.2018 and 31.12.2018 was accomplished. Our parameters were divided into sub-groups according to being within and above the reference ranges. The clinical application of tumor markers can be divided into 4 groups: screening, diagnostic confirmation, prognosis, and monitoring of recurrence. Internal Medicine, Gastroenterology, Endocrine Diseases, Chest Diseases, General Surgery, Gynecology and Obstetrics and Medical Oncology have made requests.
RESULTS:Total requests were 1420 for CEA, 671 for CA15-3, 868 for CA 19-9, and 585 for CA 125. A significant difference between genders for CEA and CA 125 was determined ($p <0.001$ and p: 0.033, respectively). 312 (22%) of CEA, 202 (30.1%) of CA 15-3, 204 (23.5%) of CA 19-9, and 113 (19, 3%) of CA 125 requests were above the reference ranges. Significant positive correlations were determined between age and tumor markers of CEA, CA 15-3, and CA 19-9 (r: 0,262, $p<0,001$; r: 0,096, p: 0,013; r: 0,090, p: 0,008; respectively). Preliminary diagnoses were nonspecific pain, acute vaginitis, anemia, anxiety disorder, dyspepsia, neoplasm and thyroid disorders.
CONCLUSIONS:This study shows that many outpatient clinics have made excessive amount of tumor marker requests incompatible with preliminary diagnosis suggesting overutilization. This situation causes cost and workload.
Keywords: Inappropriate Test Request, Oncology, Neoplasm Tumor Markers

O-012
Detection of preanalytical errors in blood gas analysis

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OBJECTIVES:Blood gas analysis is an urgent test needs to be studied in a short time interval for its preanalytical instability and there is still no consensus about the storage temperature. The aim of this study was to determine the effect of air bubbles in the blood gas injectors and different temperature conditions on the results.
MATERIALS and METHODS:Arterial blood was collected from 20 patients in intensive care unit into lithium heparin syringes from their catheter. The samples were grouped as; room temperature, room temperature plus air bubble, +4 degrees, and +4 degree plus air bubble. Blood gas analyses were performed by a potentiometric method using a blood gas analyzer ABL 800 (Radiometer,Copenhagen,Denmark) within 5 minutes (baseline) and at 30, 60, 90 and 120 minutes. Results were compared with baseline statistically with paired

samples t-test or Wilcoxon signed rank test with post hoc Bonferroni correction ($p<0.0125$), and evaluated clinically according to the desirable bias.
RESULTS:PCO2 results were increased significantly in all study groups. PO2 levels were unaffected at room temperature up to 60', but found as increased at 30' when cooled. PH levels were all in acceptable limits. HCO3 was stable up to 90', and SaO2 levels were affected less than 1% in all groups.
CONCLUSIONS:Cooling the arterial blood sample in plastic syringe is inappropriate for pO2 analysis, however, it was found as stable up to 60' at room temperature. In conclusion, room temperature is better than cooling samples in plastic syringes for arterial blood gas analysis.
Keywords: blood gas, preanalytical errors

O-013
The Effect of Hemolysis and Storage Conditions on Insulin Stability

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OBJECTIVES:Biochemical or spectrophotometric measurements are known to be more affected by hemolysis when compared to immunochemical analysis. This situation can often lead to less consideration on immunochemical assays. Threshold values at which hemolysis affects immunochemical tests are indicated in our kit inserts, but there is no value related to insulin. Therefore, the aim of this study is to determine the hemolysis threshold for insulin and the effect of storage conditions on serum insulin stability.
MATERIALS and METHODS:Serum pools were formed from the samples of the routine laboratory. Serum samples of equal volume were transferred to seven tubes. The tubes were designed as only serum in the first tube, serum + assay diluent in the second tube, and serum + hemolysate in the 3-7 tubes which correspond to 50,100, 200, 400 and 800, respectively hemolysis index. In addition, insulin levels were measured in the patient samples with <20 (n = 10), 20-50 (n=10), 50-100 (n=10) and 100-200 (n=10) hemolysis index immediately and after 8 hours at room temperature.
RESULTS:Negative bias was detected as 10% in the samples with below 200 mg/dL hemolysis index which were analysed immediately after centrifugation. Negative bias was determined as <10%, 27.6% and 29.5% in the samples <20, 20-50 and 50-100 hemolysis index, respectively which stayed for 8 hours at room temperature.
CONCLUSIONS:Hemolysis index should be considered when reporting insulin levels. Insulin analysis is not suitable for hemolysed serum samples that have waited 8 hours at room temperature.
Keywords: Hemolysis, insulin, stability

O-014
The effect of hemolysis and storage conditions on insulin stability

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OBJECTIVES:Interferences are the most serious limitations of immunoassays which are one of the most common measurement methods in clinical chemistry. According to the measurement principle of immunoassay, sources of interference may vary from antibodies, to vitamins, drugs and endogenous molecules. Unconjugated estriol (uE3) assay is an important component of second trimester screening and negative interference of uE3 assay may lead to false Down Syndrome and even Smith Lemli Opitz Syndrome risk. The aim of this case report is to present a case series of 70 patients with falsely low uE3 results from Beckman Coulter DxI 800 instrument.
MATERIALS and METHODS:Increase in number of low uE3 results (<0.3 ng/mL) led us to confirm the results. We started to dilute samples ½ and 1/5 any sample with the result below 0.3 ng/mL. Recovery results above 150% were considered as interfered samples. 1500 patient samples were screened to detect negative interference in uE3 assay within two years period. Samples with suspicion of interference were furtherly investigated in Beckman Coulter Laboratories (Marseille, France).
RESULTS:70 samples were found to be confirmed to be affected by interference which was cleared by scavenger ALP. Interfered results were below 0.56 ng/mL. Recovery results varied from 150 to 600%.
CONCLUSIONS:This is the first case series of negative uE3 interference. It was speculated that scavenger ALP molecules bind to endogenous ALP antibodies. ALP is the conjugate of manufacturer's uE3 kit and any molecule interfering with ALP affects uE3 kit.
Keywords: Interference, immunoassay, uE3

O-015
What if all is well except insulin: A macroinsulin case report

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OBJECTIVES:Macroinsulin is a larger molecule of insulin comprised of insulin and insulin antibody (IA). This phenomenon is rare and generally due to exogenous insulin therapy. The aim of this case report is to present a patient with macroinsulin who has never had exogenous insulin.
MATERIALS and METHODS:A 75-year old male was admitted to our laboratory for routine check-up. All test results including fasting and postprandial glucose levels and HbA1c were within age-specific reference intervals, except for fasting and postprandial insulin levels (110.80 µIU/mL and 163.80 µIU/mL, respectively). He had no history of insulin resistance or diabetes mellitus. His fasting and postprandial C-peptide, islet antibody, glutamic acid decarboxylase antibody levels were normal However, insulin antibody level was found to be eight fold higher than the upper limit. To prove the reason for elevated insulin, polyethylen glycol (PEG) solution is used to precipitate the insulin-IA complexes and serum insulin was re-analysed from the supernatant. Two different patients' sera with high insulin levels were also treated with PEG as control study.
RESULTS:Result of insulin in PEG-treated patient sample has been found to be decreased from 110.80 to 19.20 µIU/mL (~80%) of the first insulin measurement. Insulin results of the PEG-treated control sera were found to be similar with native sera.
CONCLUSIONS:Discrepantly high results of insulin with normal C-peptide has to be furtherly investigated with IA measurement and re-analysis of from PEG-treated serum. Insulin-IA complexes thought to be responsible for the elongated half-life of insulin in the circulation.
Keywords: Insulin, macroinsulin, insulin antibody, antigen-antibody complex, interference

O-016
Comparison of biochemical analytes in different blood collection tubes and evaluation of stability

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OBJECTIVES:In this study, we compared 3 different clot-activator gel tubes to a glass reference tube and evaluated the effect of storage time on 31 different biochemical analytes.
MATERIALS and METHODS:Blood samples were collected in 4 types of tubes: an additive- and gel-free glass tube and three different clot-activator tubes containing gel (Samplix, Vacuette, and Vacutainer). In addition to comparison with the glass tube, stability analyses were performed in Samplix, Vacuette, and Vacutainer tubes after storage for 48 hours at +4°C. Clinically important differences were evaluated using the Ricos desirable specifications for bias based on biological variation.
RESULTS:Clinically important differences were found for albumin(bias%, -2.39), sodium(-0.29), potassium(2.35) and magnesium(2.78) in Samplix; for sodium(-0.27), potassium (2.82), lactate dehydrogenase(4.47) and magnesium(2.46) in Vacuette; and for calcium(-1.56), chloride(0.66), potassium(3.54), lactate dehydrogenase(9.11) and sodium(0.38) in Vacutainer. At the end of the 48 hours, analytes that demonstrated instability were albumin(-3.13), chloride(1.01), potassium(2.69), sodium(0.54), and total protein (1.95) in Samplix; albumin(-6.45), Cl(1.11), potassium(2.06), and sodium(0.84) in Vacuette; and albumin(-4.57), calcium(1.28), chloride(0.64), free T3(-8.87), glucose(2.76), potassium(2.19), sodium(0.65), and total protein(2.15) in Vacutainer.

CONCLUSIONS:Various blood collection tubes (BCTs) in different contents may cause clinically important differences in the test results. Therefore, each laboratory should verify the reference range transfer or create its own reference range before using a new BCT. It should be also considered that in cases where analysis cannot be completed immediately after blood sampling, not all clinical chemistry or immunological test analytes can maintain their stability in BCTs up to 48 hours.
Keywords: Blood collection tube, Serum, Stability

O-017
Elevated high sensitivity troponin in the absence of coronary artery disease: A case report

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OBJECTIVES:The Joint European Society of Cardiology/American College of Cardiology committee for the redefinition of myocardial infarction (MI) states that troponins are the preferred cardiac marker for detecting myocardial injury. There are a few important non-ACS causes of cardiac troponin elevation that require immediate attention and treatment.
MATERIALS and METHODS:This is a case of a 56-year-old female came to the emergency department complaining of shortness of breath and lightheadedness. She denied any chest pain, nausea or vomiting. ECG showed sinus rhythm with minimal ST-T deviation,and chest X-ray showed no acute process. Routine biochemistry tests were within normal limits, except for hsTnT of 22.05 ng/L((0 – 14 ng/L) and Hgb of 7,1 g/dL (12,5-16 g/dL). Repeat test of hsTnT (after 1 hr,and 6 hr) respectively; 30 ng/L and 25 ng/L
RESULTS:She was diagnosed to have anemia and was given one unit of blood. Anemia was considered to be the cause of elevated troponin levels. Severe aortic stenosis was detected in the echo performed 6 months later, ECG showed sinus rhythm with ventricular HPT findings. NT Pro-BNP was found to be 1952 pg/mL (significant > 900 pg/mL)
CONCLUSIONS:Coexistence of aortic stenosis and anemia explains increased troponin values. This case report confirms that BNP test can be used as an early marker in cardiac function monitoring. It also shows that moderate elevations in troponin levels can be an indicator of non cardiac MI The importance of cardiac function tests should be discussed for more frequent follow-up of rheumatic valve patients.
Keywords: troponin, anemia, aort stenosis, proBNP

O-018
Serum separation problem on gel tubes: Is it a problem or a clue of some clinical conditions?

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OBJECTIVES:Serum separator tubes which contain separator gels are widely used by many laboratories. The gel forms a physical barrier between the cellular elements of the blood and serum. We report a case with serum separation problem in a patient hospitalized for recurrent epistaxis and regulation of hypertension.
MATERIALS and METHODS:The patient was 85 years old female followed in internal medicine department. The blood sample was collected into a BD Vacutainer SST II Advance (Becton Dickinson, NJ, USA) containing serum separator gel tube. After centrifugation, pipetting error alerts were triggered and we observed that the gel did not constitute a separating barrier and the serum did not occur. We evaluated that the underlying causes of this condition could be multiple myeloma, any radio-contrast dye usage or dialysis catheters. A second sample was collected into BD Vacutainer CAT (Clot Activator Tube) and the serum did not occur, also. A subsequent blood sample was collected into BD Vacutainer Barricor LH Plasma tube which has a mechanical separator and after centrifugation biochemical analyses were performed with plasma.
RESULTS:The analysis resulted a highly increased IgG (111 g/L (reference interval 7.51-15.6 g/L)) and total protein (120.32 g/L (reference interval 66-83 g/L)) concentration. With these results a bone marrow examination was performed and the patient was diagnosed with Multiple Myeloma.
CONCLUSIONS:This case report confirms that laboratories and tube manufacturers should be aware of the limitation of the separator gel tubes in patients with high plasma density and its effects on test results.
Keywords: separator gel, blood collection tube, hyperproteinemia

O-019
Evaluation of inflammatory status with procalcitonin and neopterin in healthy overweight and obese adults based on waist-hip ratio

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OBJECTIVES:In this study we aimed to evaluate the role of hs-CRP, Procalcitonin (PCT) and neopterin as inflammatory markers in the diagnosis of chronic low-grade inflammation associated with obesity.
MATERIALS and METHODS:67 obese, overweight and healthy adults with a mean age of 41.1±10 years were included in the study. All participants were divided into two groups according to waist hip ratio (<0.9 Group-A, ≥ 0.9 Group-B) and three groups according to body mass index (BMI) (< 25 Group- 1, 25-29 Group-2, ≥30 Group-3). Hs-CRP, PCT, neopterin levels of the groups were compared between the groups. Lipid profile and blood glucose levels also evaluated.
RESULTS:There was no significant difference in CRP, NP, PCT between the groups formed according to waist hip ratio (p> 0.05). In BMI groups, CRP levels were found to be elevated with obesity in BMI groups. There was a difference between Group-1 and Group-2 and Group-2 and Group-3, but they were not significant. The difference between Group 1 and Group 3 was significan t(p <0.05). There was no difference in NP levels between the groups (p> 0.05). In the PCT levels, there were statistically significant results between Group-1 and Group-2 and between Group-1 and Group-3, but no difference was found between Group 2 and Group 3(p> 0.05).
CONCLUSIONS:It was shown that the increase in total fat mass in the body may lead to an increase in inflammation markers. However, it was concluded that this difference may be more closely related to the degree of obesity rather than fat distribution
Keywords: Obesity, Inflammation, Hs-CRP, Procalcitonin, Neopterin

O-021
Simultaneous determination, quantitation and validation of the most used benzodiazepines in urine

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OBJECTIVES:A single method for confirmation and quantitation of a panel of commonly prescribed benzodiazepines and metabolites, flunitrozepam, clonazepam, diazepam, lorazepam, oxazepam, bromozepam, clobazam, flurazepam, midazolam, and triazolam was developed for urine samples.
MATERIALS and METHODS:Quantitation was by liquid chromatography tandem-mass spectrometry (LC-MS-MS) using a AB-Sciex 4500 Q-TRAP system. The instrument was operated in multiple reaction monitoring mode with an electrospray ionization source in positive ionization mode. Deuterated analogues were included as internal standards for all 10 analytes. The method was evaluated for recovery, bias, imprecision, linearity, analytical range, carryover, and matrix effect.
RESULTS:The measurement of calibration dependence allowed to determine the extent of linearity in the concentration range from 12.5 to 500 ng/ml for all benzodiazepines except midazolam flunitrozepam, clonazepam, oxazepam, clobazam, which were linear through 25 to 500 ng/ml with acceptable coefficients of determination (r2>0.99). For all analytes at concentration levels 12.5, 300 and

500 ng/ml (n=5, 3 days) BIAS and precision (within-run and between-run) were established in the range 2.7 to 4.10 and 0.83 to 9.90, respectively. Detection limits (LOD) ranged at 0.95-7.27 ng/ml and quantification limits (LOQ) at 2.89-22.04 ng/ml for all analytes, which were both highest for Clobazam. No carry-over was observed after the injection of 500 ppb certificated reference material. For matrix effect evaluation, relative recovery was established between 91% to 97%, except midazolam (absolute recovery 88%, relative recovery 76%).
CONCLUSIONS:The applicability of a simple LC-MS/MS method was proven by for analyzing authentic urine samples and third party external quality samples in urine matrix.
Keywords: Benzodiazepines, Validation, LC–MS/MS method

O-022
Association of NUCB2/Nesfatin-1 gene polymorphism with obstructive sleep apnea severity

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OBJECTIVES:Obstructive sleep apnea syndrome (OSAS) is characterized by repetitive obstruction of upper respiratory tract and associated with decrease in blood oxygen saturation. Inflammation is involved in the mechanism of obstructive sleep apnea syndrome. The aim of the study was to determine distribution of NUCB2 genotypes of nesfatin-1 in OSAS.
MATERIALS and METHODS:38 OSAS and 12 healthy subjects were enrolled in this study. Individuals were seperated into four groups (mild, moderate, severe and normal) by standard polysomnography, according to the apnea-hypopnea index. Chronic inflammatory diseases, psychiatric diseases, malignant diseases and neurogenic diseases, were excluded from the study. After sampling, DNA isolation and Real-Time PCR were performed.
RESULTS:The mean age of paticipants were 52.02 ± 10.40 years. %18 of volunteers were male and %82 of volunteers were female. Control group consist of 12 (%24) individuals had an AHI<5. NUCB2 gene polymorphisms were compared with sleep scores (severe, moderate, mild and control). There was a significant relationship between rs2634462 polymorphism and sleep scores (p=0.002). There was no relationship between rs1330 A/G, rs214101 C/T, rs757081 C/G NUCB2 gene polymorphisms and sleep scores.
CONCLUSIONS:It was found that individuals with homozygous C/C genotype of the rs2634462 polymorphism had significantly more severe OSAS compared to individuals with other genotypes. rs2634462 polymorphism of nesfatin-1 may be a new biomarker in predicting the presence of OSAS.
Keywords: NUCB2 gene, Obstructive Sleep Apnea Syndrome, Polymorphism

O-023
The effect of lycopene on autophagy in fluoride toxicity in kidney cells

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OBJECTIVES:The aim of this study was to investigate the effect of lycopene on autophagic pathway against toxicity induced by sodium fluoride in renal cell line (NRK-52E).
MATERIALS and METHODS:Cells were grown in vitro by regular passages. The IC50 value of NaF and the proliferative concentration of lycopene were determined by MTT. In the study, 4 groups were formed as control (K), fluorine (F), lycopene (L) and fluorine lycopene (FL). 24 hours after the application of fluorine and lycopene to the cells at the specified concentrations, RNA isolation and cDNA synthesis were performed and expression of the autophagic genes was determined by RT-PCR.
RESULTS:The proliferation enhancing concentration of lycopene (1 µM) and the IC 50 concentration of NaF (3200 µM) at 24 h were found. In the FL and F groups, Sqstm 1 expression increased 19 and 9 times, Atg5, 9 and 5.5 times, Map11c3a 6 and 5 times, respectively. There was no significant change in other genes.
CONCLUSIONS:As a result, it was determined that the fluoride given at IC50 concentration affects the autophagic genes studied and the highest increase occurred in Sqstm 1. It can be concluded that lycopene given alone does not alter the genes much, and in the FL group, fluorine may increase autophagy by inhibiting the proliferative effect of lycopene.
Keywords: fluorine, cell culture, lycopene, autophagy

O-025
The relationship between WNT signaling activity and organ attitudes in scleroderma disease sub-groups

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OBJECTIVES:Scleroderma is a chronic inflammatory autoimmune disease characterized by fibrosis in the skin and internal organs.The relationship between SCC type,stage,pathogenesis,organ involvement and WNT gene family has not been identified yet.We aimed to show the relationship of WNT gene family and antagonists in development of SSC subtypes of disease and different organ involvement.
MATERIALS and METHODS:The study included 85 patients with SSC and 77 controls.The gene expressions & protein levels of the WNT family and antagonists were analyzed from blood samples. The qPCR method was used for WNT gene expression levels. WNT antagonists protein levels were determined by ELISA method. The relationship between these parameters and disease stage, type and organ involvement was evaluated.
RESULTS:There was a significant increase in WNT-1, WNT-10b, WNT-2, and WNT-6 genes in the SCC group.Axin-2 is decreased. DKK-1 and Kremen protein expressions are decreased in scleroderma. There was a significant difference between WNT-3a and WNT-10a gene expression among patients with generalized scleroderma and limited SCC.WNT-3a and WNT-10a gene expression increased in generalized scleroderma. WNT-1, WNT-2 and AXIN-2 gene expression increased significantly in PAH positive SCC patients.There was a positive correlation between the modified Rodnan skin score (MRS) and WNT-2 in patients with SCC.There was a significant positive correlation between total GIS involvement score and WNT-1, WNT-2, WNT-4, WNT-8a, WNT-9b in scleroderma patients. WNT-1, WNT-2, WNT-4, WNT-8a, WNT-9b gene expression expressions increased as the disease severity scale increased.
CONCLUSIONS:WNT-1 and WNT-2 were found to be high in the skin and organ involvement of scleroderma. It was found to play a role in the pathogenesis of the disease.Therefore, we identified new therapeutic targets in SCC.
Keywords: WNT signaling pathway, WNT antagonists, scleroderma, organ involvement

O-027
Towards the clinical implementation of pharmacogenetics in cardiology: Serbian experience

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OBJECTIVES:The use of pharmacogenetic testing in cardiology is rapidly expanding and constantly refining. The objective of this study was to evaluate the effect of the CYP2C19, ABCB1, PON1 and P2RY12 variants on clopidogrel pharmacodynamics and clinical outcomes in Serbian ST-elevation acute myocardial infarction (STEMI) patients undergoing primary PCI (pPCI).
MATERIALS and METHODS:One hundred and forty consecutive patients referred to pPCI for STEMI in a high-volume cath lab were enrolled in the study. Clopidogrel response was assessed with multiple electrode platelet aggregometry. The clopidogrel-metabolizing pathway SNPs used were: CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893), CYP2C19*17 (rs12248560), ABCB1 (rs1045642), PON1 (rs854560, rs662), and P2RY12 (rs2046934). The primary clinical endpoint was major adverse coronary and cardiovascular event (MACCE) defined as death, nonfatal myocardial infarction, ischemia-driven revascularization and stroke. The secondary clinical endpoint was bleeding occurrence. Bleeding was defined according to the Bleeding Academic Research Consortium definition. The follow-up period was one year.
RESULTS:One-year MACCE was 12.9%. All alleles and genotype proportions were found to be in Hardy–Weinberg equilibrium (p>0.05). Among the SNPs tested, only CYP2C19*17 was significantly associated with MACCE, but not with clopidogrel response. Our results did not find CYP2C19*2 or CYP2C19*3 to be significantly associated with MACCE. Bleeding was not significantly different across the CYP2C19, ABCB1, P2RY12 and PON1 genotype groups.
CONCLUSIONS:In clopidogrel-treated patients with STEMI undergoing pPCI, CYP2C19*17 was independently associated with an increased risk of MACCE independent of clopidogrel responsiveness. The bleeding risk does not appear to be explained by CYP2C19 genotype.
Keywords: clopidogrel, pharmacogenetics, STEMI

O-030
Inhibitory effect of glyphosate on butyrylcholinesterase and acetylcholinesterase activity

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OBJECTIVES:Herbicide glyphosate (N-phosphonomethyl glycine) began to be used in 1974 for weed control in agricultural production areas. In 2015, the World Health Organization(WHO) classified glyphosate as a possible carcinogen for humans. Increased glyphosate use was correlated with various types of cancer, Alzheimer, autism and Parkinson's diseases. Cholinesterase enzymes are found in large amounts in the brain and also inhibit organophosphate poisoning. Therefore, we aimed to make a preliminary study on the effect of glyphosate on cholinesterase enzyme types.
MATERIALS and METHODS:Inhibition effects of glyphosate at various concentrations (282 mg/L, 28.2mg/L, 2.8mg/L, 0.7mg/L) of acetylcholinesterase in human erythrocytes and butyrylcholinesterase in human plasma were examined for 10 min, 30 min and 1 hour preincubation periods. Colorimetric kinetic measurements of cholinesterases were performed using the Ellman's method.
RESULTS:The decrease in measured butyrylcholinesterase enzyme activity was measured in the different glyphosate concentrations depend on preincubation periods. The most important decrease was observed at butyrylcholinesterase with 31% loss of activity at a concentration of 282mg/L glyphosate in 1 hour preincubation. Acetylcholinesterase enzyme activity decreased 11% activation at a high concentration of glyphosate 282mg /L, while a decrease in time-dependent on preincubations was not measured. There was no decrease in acetylcholinesterase activity at other glyphosate concentrations.
CONCLUSIONS:Some articles have controversial statements about the inhibition of glyphosate on cholinesterases. Because of the effect of glyphosate in neurological diseases, we investigated the interaction of both enzymes with glyphosate in vitro conditions. It was concluded that prolonged exposure to glyphosate may cause pathological findings.
Keywords: Glyphosate, Acetylcholinesterase, Butyrylcholinesterase, Ellman's Method

O-031
Evaluation of Roche Accu-Chek Inform II Glucose test strip system in the hospital setting

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OBJECTIVES:Glucometers are widely used in hospital wards as a practical tool to get immediate results concerning the patient's glucose status. Accurate bedside glucose measurements are of paramount importance in the evaluation and treatment of diabetic patients.
MATERIALS and METHODS:According to our institutional quality assurance policy, weekly venous blood samples are analyzed on the sampling by Glucometer (Roche Accu-Chek Inform II, performed by nurses) and the Laboratory (Roche Cobas c6000, c501). 246 patients' venous blood glucose results (Glucometer and Laboratory) were analyzed. Wilcoxon test was performed on paired venous blood sample results. Performance according to ISO 15197 2013 standard including consensus error grid analysis was investigated.
RESULTS:Glucometer and Laboratory median glucose (mg/dl) levels were 136,5 (range: 75 – 461) and 134,5 (range: 71 – 441) respectively (p=0,0061, Wilcoxon). Coefficient of variation of paired results was 7,7 % (95 % CI: 5,9 – 9,2). 91,1 % of glucometer glucose results (224/246) were within allowed limits according to ISO 15197 2013. This performance did not meet the ISO 15197 2013 standard which requires that at least 95 % of results should be within limits. Nevertheless, all 22 patients glucose data beyond the standard's limits were in the zones of a (86 %) and b (14 %) of consensus error grid area. Thus fulfilling standard's 2nd necessity at a rate of 100% (at least 99% required).
CONCLUSIONS:According to our routine quality control performance data, the Roche Accu-Chek Inform II glucometer system is clinically useful for professional use in health care institutions.
Keywords: venous glucose, Accu-chek inform II, Cobas 6000, ISO 15197 2013, consensus error grid

O-032
Evaluation of urine drug screening test results between 2016-2018 years in Kanuni Education and Research Hospital Laboratory

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OBJECTIVES:Drug abuse one of the most important health problems in the world and unfortunately it is rapidly increasing in Turkey as well. In order to establish valid policies on this issue, the initial step is to define the extent of this problem by determining the prevalence of use. Further information on the frequency of substance use is essential for preventive studies. We planned this study to determine which drugs are analyzed more widely in Trabzon and to evaluate their distribution according to age and sex, and therefore to collect and present data to take measures.
MATERIALS and METHODS:Urine drug screening tests (Amphetamine, Benzodiazepine, MDMA-Ectacy, Barbiturate, THC-Cannabis, Cocaine, Bonzai-Spice1 / Spice2, Opiate, Buprenorphine) were evaluated with retrospective LIS data and the results were determined as positive by age and sex.
RESULTS:Cannabis (THC) was commonly used banned substance in 16% of patients admitted between 2016-2018, followed Benzodiazepine (9.97%) and Buprenofrin (8.93%). Bonzai spice1 and spice2, which are thought to be widely used, are 0.045% and 0.3% respectively. The important reason for the low usage of this substance, which has increased in recent years, is the existence of product variety which limits its detection by the current method. Percentages of other substances were cocaine 0.3%, MDMA 1.76%, Opiate 1.37%, Amphetamine

2.25%, Barbiturate 0.058%, respectively. 95.61% of the user is male, 4.51% is female and the average age is around 31 years and the lowest-highest age is 14-78.
CONCLUSIONS:The increase in the frequency of substance use among women and decreasing user age raises concern. However, the research is regional and countrywide studies are needed.
Keywords: drug abuse

O-033
Pregabalin substance abuse

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OBJECTIVES:Pregabalin is an antiepileptic drug that reduces the release of excitatory neurotransmitters such as glutamate, substance P. It is approved to be used in adult patients who have peripheral neuropathic pain, fibromyalgia, epilepsy. Drug bind to the α2-δ subunit of voltage-dependent calcium channels in central nervous system. It is well known that pregabalin has some abuses potentially. Several databases have warned for overdose fatalities. Overdoses of gabapentinoids can become lethal in mixture with other psychoactive drugs, especially opioids.In this study, we aimed to reveal the abuse of pregabalin.
MATERIALS and METHODS:In our laboratory, drug analysis is carried out by mass spectrometry method with QTrap analyzer. These analysis were performed on urine samples. All results which analysed between 01.02.2019-10.07.2019 were examined retrospectively. Pregabalin positive samples were evaluated among the results. Amphetamine and its derivatives, opioids, codeine, morphine, heroin, cannabis, cocaine, benzodiazephines, synthetic cannabinoids, pregabalin, gabapentin analyzed in all samples.
RESULTS:1522 patients results evaluated. In 22.8% (347/1552) samples, pregabalin concentration was higher then 50 ng/ml. It has been found that 140 positive pregabalin results have a level of over 1000 ng/ml. Moreover, cannabis has been found on 68 samples in addition to pregabalin. In some samples it has also been found amphetamine or cocaine and multiple substance were positive with pregabalin.
CONCLUSIONS:According to our data pregabalin abuse is common. Since pregabalin can cause undesirable consequences like other addictive substances, health professionals and prescribers must be aware of this misuse potential. Laboratory professionals should be able to measure pregabalin in drug laboratories.
Keywords: Pregabalin, Lyrica, substance abuse

O-034
The protein supplements and inhibition of liver enzymes at athletes

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OBJECTIVES:The aim of study was to investigate influence of protein supplements changes on liver enzymes (ALT, AST, ɣ -GT and LD) in athletes with high and low intensity training.
MATERIALS and METHODS:The 180 male athletes were divided in three groups of subjects, athletes with high intensity training, athletes with low intensity training and the control group. We analyzed the activity of enzymes ALT, AST, ɣ -GT and LD, proteins in the urine, as well as urea and creatinine. The enzyme activity was determinated with a BS-200 Mindray machine.
RESULTS:The results of enzymes activity in vitro show that using protein supplements increase activity of these enzymes: ALT 56.68 %, AST 48.78 %, ɣ -GT 14.17 and LD 9.71 % in the serum of athletes during training comparing the athletes who do not use supplements. The mean differences between the parameters ALT, AST, ɣ -GT and LD between the groups was a statistically significant ($p < 0.05$) between subjects who use supplements and those who do not use supplements. The Man Whitney U test showed that between subjects (high intensity and low intensity training) there is a statistically significant difference between the all examined parameters, while the LD did not show a statistically significance difference ($p > 0.05$).
CONCLUSIONS:The protein supplements (Whey protein, Gainer, Isoactive, BCAA) increased activity of the enzyme ALT, AST, ɣ -GT, LD in athletes. The activity of the enzyme decreases in the serum after a seven-day break of using the protein supplements.
Keywords: liver enzymes, Whey protein, Gainer, Isoactive, BCAA

O-035
Effect of bariatric surgery on ghrelin-hepatosteatosis interaction: The Selcuk University Faculty of Medicine example

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OBJECTIVES:During the last years, bariatric surgery has become an established procedure for effective and sustainable weight loss. In the majority of patients, bariatric surgery improves liver steatosis, inflammation, and fibrosis in nonalcoholic fatty liver disease patients with obesity. The aim of our study was to investigate the effect of bariatric surgery on ghrelin-hepatosteatosis interaction in morbid obese patients.
MATERIALS and METHODS:23 patients who underwent bariatric surgery (BMI=49.27±7.46 kg/m2) in Selcuk University Faculty of Medicine Clinic of General Surgery were included in the study. Sixteen of these patients were operated with laparoscopic sleeve gastrectomy and seven were operated laparoscopic Roux-en-Y gastric bypass method. Blood samples were collected from the patients before of the operation and at 1st, 3rd, 6th months after the operation. Hepatosteatosis were performed by radiologists with ultrasonography. Ghrelin levels were studied by elisa method.
RESULTS:There was not found significant difference in ghrelin levels between preoperative and postoperative periods ($p=0.384$). The hepatosteatosis was significantly decreased at postop 1st, 3rd, 6th months compared to preop period ($p<0.05$). There was a weak, significant and negative correlations between ghrelin levels and hepatosteatosis at postoperative 1st and 3rd months.
CONCLUSIONS:As a result, bariatric surgery has improved some endocrine abnormalities, but did not show any significant difference in ghrelin levels. Significant reductions in hepatosteatosis were observed after surgery. Further studies involving ghrelin and hepatosteatosis should be perform.
Keywords: Bariatric surgery, ghrelin, hepatosteatosis

O-036
The results in two different provinces in Black Sea Region where thalassemia screening was implemented:a rare hemoglobin variant

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OBJECTIVES:We aimed for assessing the results of a thalassemia test implemented for the purpose of screening in the provinces of Amasya and Tokat and for revealing the clinical features of a rare variant type of hemoglobin in our study.
MATERIALS and METHODS:The results of n=2258 samples (55.8% males and 44.2%), sent from the provinces of Amasya and Tokat for the purpose of thalassemia(hemoglobin variant) screening between 15.10.2018-31.05.2019 were retrospectively examined in Public Health Laboratory in Central Amasya. Hemoglobin variant analysis was carried out through the method of HPLC (High Performance Liquid Chromatography) on Primus Ultra2 device. The sample was also examined on a different system for the rare hemoglobin variant (Hb Pusan) and DNA strand analysis was implemented for substantiation for Hb Pusan variant.

RESULTS:In accordance with the results regarding patients retrospectively screened, while the results of n=2170 (56.3% males and 43.7% females), patients were found to be normal; 37 patients (40.6% male and %59.4 female) with suspected of alpha thalassemia was detected), 50 patients (44% males, 56% females) with suspected of beta thalassemia was detected, hemoglobin 1 female patient with suspected of hemoglobin E variant was detected and hemoglobin Pusan variant was detected in n=1 male patient.
CONCLUSIONS:In accordance with our findings, it was discovered that frequency of beta thalassemia is higher than other types of variant in both provinces.
Keywords: thalassemia, hemoglobin variant analysis, HPLC, hemoglobin Pusan

O-037
First observation of Hemoglobin Hamilton [β11(A8)Val→Ile] in Turkey

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OBJECTIVES:Until today, approximately 60 hemoglobin variants have been identified in Turkey. One of them, Hb Hamilton, β11(A8)Val→Ile, which is a silent mutation, is the substitution of isoleucine for valine in the 11th position of the beta chain. This mutation does not change the function of the hemoglobin molecule. TheUntil today, approximately 60 hemoglobin variants have been identified in Turkey. One of them, Hb Hamilton, α2β211(A8)Val→Ile, which is a silent mutation, is the substitution of isoleucine for valine in the 11th position of the beta chain. This mutation does not change the function of the hemoglobin molecule. The variant can not be determined by cellulose acetate, starch or agar gel electrophoresis. It was discovered in an Austrian family living in Canada by Triton X-100 acid-urea polyacrylamid gel electrophoresis in 1984. Then, cord blood samples of 4581 babies born between 1985 and 1986 in Sardinia hospitals screened for Hb Hamilton using the same method. In this case study we aimed to report the first observation of Hb Hamilton in Turkey. variant cannot be determined by cellulose acetate, starch or agar gel electrophoresis. It was discovered in an Austrian family living in Canada by Triton X-100 acid-urea polyacrylamid gel electrophoresis in 1984. Then, cord blood samples of 4581 babies born between 1985 and 1986 in Sardinia hospitals screened for Hb Hamilton using the same method.
MATERIALS and METHODS:Five milliliters of blood samples from patients were collected in ethylenediaminetetraacetic acid (EDTA) vacutainers for estimation of blood count. Hemoglobin variants were characterized by high performance liquid chromatography (HPLC). Micro column method was used for the isolation of DNA samples. Screening of beta globin gene was performed by DNA sequence analysis.
RESULTS:During genetic screening of hemoglobinopathies in Diyarbakır, hemoglobin Hamilton was detected by DNA sequence analysis. Fourteen different β-thalassemia mutations and 3 abnormal hemoglobins (HbS, HbD-Punjab, Hb Hamilton) were detected in 53 adults. Hb Hamilton was seen in combination with beta-thalassemia mutation (IVS1-110 G>A).
CONCLUSIONS:The presence of Hemoglobin Hamilton was reported for the first time in Turkey. This variant could not be detected when the sample was screened by HPLC.
This project was supported by Dicle University Research Project Unit (TIP.18.001& TIP.19.008).
Keywords: Hb Hamilton, HbS, HbD-Punjab, β-thalassemia

O-038
Glanzmann thrombasthenia: A case report

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OBJECTIVES:Glanzmann's Thrombasthenia (GT) is a rare autosomal recessive disorder that affects the platelet glycoprotein IIb/IIIa (GPIIb/IIIa) complex and characterized by prolonged bleeding time. The medical history of the patient and the family history of consanguinity crucial while evaluating the patient. In most cases, bleeding symptoms apparent rapidly early in life, but diagnose of GT needs highly specialized centers. In bleeding disorders, routine coagulation tests may be normal so as differential diagnosis specific test such as platelet function and flow cytometry could be used.
MATERIALS and METHODS:A 40-year-old man, presented to the hematology department with spontaneous ecchymosis, was referred to our laboratory for investigating the bleeding disorder. In addition to routine hematological tests, platelet function tests were performed on an Aggram(Helena) agrometer and Innovance-PFA200(Siemens). A Flowcytometeric analysisis (Navios-Ex, Beckman Coulter) was used to confirm the results.
RESULTS:Patient's WBC:3.5*10^3/µL, RBC:4.96*10^5/µL, Hb:13.5g/dL, Hematocrit:40.1%, Platelet: 160*10^3/µL, PT:12.5sec, aPTT:23sec, Fibrinogen:235mg/dL. Peripheral blood smear was normal. Closure times in PFA analysis; Collagen-epinephrine>300sec and Collagen-ADP:>273sec. In aggregometry, collagen, ADP, epinephrine results were normal, while abnormal aggregation curve with ristocetin was observed. CD42a expression was normal and CD41&CD61 were reduced in flow cytometry. The patient was diagnosed with GT.
CONCLUSIONS:GT is a rare bleeding disorder. Clinical findings may vary from petechiae, gingival bleeding to severe life-threatening bleeding. Typical characteristics are long bleeding time, normal platelet counts and no peripheral blood smears. When evaluating a patient with bleeding disorder, GT should be kept in mind and further investigations should be performed.
Keywords: Glanzmann Thrombasthenia, Platelet glycoprotein IIb/IIIa, Inherited platelet disorder, Platelet function test

O-040
Determination of electrochemical behaviour of glucose-6-phosphate dehydrogenase by biosensor

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OBJECTIVES:Glucose-6-phosphate dehydrogenase (G6PD) has a housekeeping role in all cells and is particularly critical to the integrity and functioning of red blood cells. In this study, the activity of G6PD on bioactive surface was investigated. For this purpose, potentiodynamic polarization curves and cyclic voltammetry measurements were used. The surface morphology of the biosensor was investigated by scanning electron microscopy (SEM).
MATERIALS and METHODS:BSA/gelatin and gluteraldehyde was used as a polymer and cross-linking agent, respectively. Cyclic voltammetry measurements were performed using 3-electrode sensing system in 5mM pH 7.0 phosphate buffer. Gold electrode as working electrode, Ag/AgCl as reference electrode, platinum as counter electrode was used. Furthermore, SEM images of enzyme immobilized and enzyme free polymer on electrode surface were compared.
RESULTS:Our study showed that G6PD enzyme carried out the oxidation reaction with 2.5µA lower energy. In addition, current flowing reduced by enzyme showed that it complied with the OHM law. From the results obtained G6PD, electrochemical reaction on bioactive surface was found to be effective by reducing energy requirements. Parallel with this information, SEM images also showed surface differences of the enzyme immobilized and enzyme free polymer.
CONCLUSIONS:These potentiometric measurements showed how much enzyme reduced activation energy. Since the main function of enzymes was to

lower the activation energy, here we have confirmed that the enzyme worked and therefore the immobilization application was successful. Thus, we have completed the preliminary study of a sensitive method that we planned. In further studies, the responses of G6PD enzyme with natural substrates will be evaluated.
Keywords: Biosensor, G6PD Enzyme, Immobilization, Polarization, SEM.

O-041
Investigation of the effect of glyphosate on G6PD activity in *in vitro* conditions

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OBJECTIVES:Glucose 6-phosphate dehydrogenase (G6PD) is a key and rate limiting enzyme in the pentose phosphate pathway. Many substances, especially herbicides, can inhibit G6PD enzyme activity *in vitro* and *in vivo*. Glyphosate is the most widely used herbicide in worldwide. The aim of this study was to investigate the effect of glyphosate on the erythrocyte G6PD enzyme *in vitro* due to the structural similarity of the substrate to the phosphate group of Glucose-6 phosphate.
MATERIALS and METHODS:In this study, the effect of different glyphosate concentrations (282 mg/L, 28.2mg/L, 2.8mg/L, 0.7 mg/L) on G6PD enzyme activity was investigated. Hemolysate was prepared from erythrocytes obtained from healthy, adult male individuals as samples. Enzyme activity was measured using the Beutler method.
RESULTS:Inhibition percentages for glyphosate administration at different concentrations with 0/10/30. minute incubation; 0.1 M glyphosate 21%, 24.8% and 26% respectively, 0.01 M glyphosate 20.8%, 22% and 11.8%, 0.001 M glyphosate 1.5%, 9.78% and 10%, 0.0005 M glyphosate 1.3%, 6.25% and 8%, 0.00025 respectively M glyphosate was observed as 0.07%, 2.8% and 2.14%. The increase in activity observed in the 60 minute incubation suggests that the enzyme is a semi-competitive inhibitor.
CONCLUSIONS:According to the results of our studies, it is seen that inhibition increases due to the increase in the glyphosate concentration and incubation time. Due to the high number of individuals with G6PD enzyme deficiency and increased exposure to glyphosate in the Çukurova region, our study is very important in terms of preventing health problems that may occur.
Keywords: Glyphosate, G6PD, enzyme inhibition

O-042
The evaluation of microtubes' compatibility to automated process for complete blood count

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OBJECTIVES:Newborns and occasionally patients with malignancy develop anemia due to iatrogenic blood loss based on phlebotomy for blood tests, including complete blood count (CBC). Reducing the need for blood transfusion and thus avoiding the associated risks due to frequent phlebotomy involves limiting blood sampling and the use of micro tubes as primary tubes in automated analyzers. It is aimed to compare microtubes with the vacuum tubes of the same brand in terms of accuracy and ease of use.
MATERIALS and METHODS:Venous blood samples were taken from 40 in-patients and collected in three different brand microtubes/evacuated tubes pairs (Microtainer MAP-0.5 mL/Vacutainer-2.0 mL;Becton, Dickinson and Company,USA)(Microvette-0.5 mL/S-Monovette-2.6 mL;Sarstedt Ag & Co. KG,Germany)(MiniCollect Complete-0.5 mL/Vacuette-2.0 mL;Greiner Bio-One GmbH,Austria). All tubes contained K2EDTA except Microvette. White blood cell(WBC), red blood cell(RBC), hemoglobin, platelet(PLT) were analyzed using a CBC analyzer (DxH 800, Beckman Coulter Inc., USA).
RESULTS:The bias (%) of WBC, RBC, hemoglobin, and PLT parameters between a microtube and a standard tube for each brand was calculated and presented as follows: Microtainer MAP vs. Vacutainer -0.41, 0.52, 0.46, and -1.34; Microvette vs. S-Monovette -1.09, 0.61, 0.51, and -1.49; MiniCollect Complete vs. and Vacuette, -0.24, 0.34, 0.05, and -0.89. All bias calculations were within the desirable limits based on the Ricos' biological variation data.
CONCLUSIONS:According to the results, laboratories using these CBC tubes can limit blood sampling in their efforts to reduce iatrogenic blood loss, by providing and using micro-volume pairs of the same brand without extra effort, especially in patients requiring frequent phlebotomy.
Keywords: blood cell count, blood specimen collection, laboratory automation, blood volume

O-043
Design of a new biosensor for the determination of ferric iron in blood

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OBJECTIVES:Iron is an element that is necessary for life but can damage the organism if it is present in excess. Iron performs many important functions in the body. Iron deficiency is the most common nutritional deficiency and the leading cause of anemia in the world. In this study, we aimed to design a biosensor for the quantitative determination of Fe^{3+} in a short time and at an affordable cost.
MATERIALS and METHODS:The bioactive layer was prepared by immobilizing hydrogen peroxidase enzymes on the gold electrode with bovin serum albümin (BSA), gelatin, glutaraldehyde with the help of UV light. To ensure separation of ferric iron (Fe^{3+}) in the serum from the transferrin, acetate buffer having a pH of 5.0 was preferred. Hydroxylamine hydrochloride was then used to reduce the ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). Ferrous iron (Fe^{2+}) which is produced as a result of the reduction was measured by the reaction of hydrogen peroxidase enzyme.
RESULTS:The response current in the range of 0.2 and 1.4 V was performed on a cyclic voltammogram at a scanning rate of 0.06 V/s. Enzymatic reaction rate decreased as substrate concentration increased in an environment where all parameters were constant.
CONCLUSIONS:In determining optimum operating conditions, acetate buffer was determined at pH 5.0 and 200 mM concentration, scanning speed was 0.06 V/s and temperature was determined as 40 °C. In this study, the best measurement was obtained with gold electrode immobilized with an enzyme concentration of 0.5 mg/ml was used.
Keywords: biosensor, hydrogen peroxidase, ferric iron

O-044
Correlation between LUC % and thyroid function tests

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OBJECTIVES:In this study, we aimed to determine the correlation between thirteen hemogram parameters and thyroid stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3).
MATERIALS and METHODS:A retrospective study was performed on 18 013 patients' data who all presented with thyroid pathologies. Laboratory results of TSH, T4 and T3 blood levels, white blood cells (WBCs), large unprotected cells (LUC), LUC%, neutrophil count, neutrophil percentage, lymphocyte count, lymphocyte percentage, monocyte count, monocyte percentage, eosinophil count, eosinophil percentage, basophil count, basophil percentage were analyzed. Blood samples were collected by venepuncture and anticoagulated with dipotassium ethylenediamine tetra-acetic acid (EDTA) and hematology parameters were measured within 1 h of collection on a Siemens ADVIA 2120i hematology analyzer (Siemens Healthcare Diagnostics, Germany). Thyroid function tests were measured by using the ADVIA Centaur XP analyzer (Siemens Healthcare Diagnostics, Germany). Normality tests were performed using single-sample Kolmogorov-Smirnov test using SPSS 18.0 for all data. Correlation analysis was performed using the Pearson method.

RESULTS:The relationship between TSH, free T3, free T4 and LUC % were r = -0.102, p = 0.001; r = 0.210, p <0.001; r = 0.127, p <0.001; respectively.
CONCLUSIONS:It can be proposed that LUC% count is weakly but significantly associated with thyroid hormon levels observed.
Keywords: inflammatuary marker; LUC%; thyroid function tests

O-045
The effect of Rhamnetine against to ischemia-reperfusion injury in the kidney

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OBJECTIVES:The purpose of this study was to investigate the possible protective effect of Rhamnetin, as a potent antioxidant on I/R-induced renal injury in rats.
MATERIALS and METHODS:We used 28 male wistar albino rat weight 200-250 g in this research. The animals were randomly divided into 4 groups. Each experimental group was consisted of seven animals. Rats were subjected to 45 min of renal pedicle occlusion followed by reperfusion. Control Group (C): Ischemia/reperfusion was not performed to animals. Rhamnetin Group (R): 100 mg/kg Rhamnetin was administered i.p 30 min prior to ischemia and immediately before the reperfusion period. Ischaemia/Reperfusion Group (I/R): Rats were subjected to 45 min of renal pedicle occlusion followed by 24 hours reperfusion. Rhamnetin+Ischemia/Reperfusion Group (R+I/R): Rhamnetin (100 mg/kg i.p) was administered 30 min prior to ischemia and immediately before the reperfusion period. Rats were subjected to 45 min of renal pedicle occlusion followed by 24 hours reperfusion.
RESULTS:MDA levels were found to be significantly increased whereas SOD and GST enzyme activities were found to be significantly decreased in I/R ($p<0.05$). However, there were no significantly differences in CAT activities and between the C and I/R groups. While GST activities were significantly elevated in R+I/R group compared to control group, MDA levels were significantly decreased.
CONCLUSIONS:These results show that treatment with Rhamnetin may prevent the kidney damages due to ischaemia result in increasing oxidant stres peroxidation damages further. This study suggests that Rhamnetin may be a effective antioxidant agent.
Keywords: Rat, Rhamnetin, ischemia, reperfusion

O-046
The protective effect of resveratrol against cyclosporine A-induced oxidative stress and hepatotoxicity

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OBJECTIVES:The immunosuppressive agent cyclosporine A (CsA) has hepatotoxic potential. Increased reactive oxygen species (ROS) formation is among the causes leading to hepatotoxicity. In this study, we aimed to investigate the effect of resveratrol (RES) treatment on CsA-induced oxidative stress and hepatotoxicity in rats.
MATERIALS and METHODS:Rats were treated with RES (10 mg/kg/day; i.p.) for 14 days. CsA (25 mg/kg/day; s.c.) was given during the last 7 days together with RES. Determinations of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities together with hepatic histopathological examinations were performed. ROS, thiobarbituric acid reactive substances (TBARS), advanced oxidation protein products (AOPP), ferric reducing antioxidant power (FRAP), and glutathione (GSH) levels as well as superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities were measured in the liver tissue.
RESULTS:CsA treatment increased hepatic ROS, TBARS, and AOPP levels significantly as compared to the control group. Although hepatic GSH levels and SOD activity did not alter, FRAP level decreased and GSH-Px activity increased significantly in CsA treated rats. CsA also caused degeneration in hepatocytes and sinusoidal spaces. RES treatment ameliorated histopathological changes and decreased hepatic ROS, TBARS and AOPP levels significantly. However, it did not change serum ALT and AST activities as well as hepatic antioxidant parameters in CsA-treated rats.
CONCLUSIONS:RES does seem to have a protective effect on CsA-induced hepatotoxicity by reducing oxidative stress. Considering the immunosuppressive and hepatoprotective efficiency of RES, the combined use of CsA and RES may be useful in hepatic transplantation therapy by reducing hepatotoxicity and increasing the immunosuppressive effect of CsA.
Keywords: Cyclosporine, resveratrol, hepatotoxicity, oxidative stress

O-047
Thiol/Disulphide balance and Ischemia-modified albumin levels in female with iron deficiency anemia

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OBJECTIVES:Iron deficiency and harmful effects of iron deficiency anemia; it develops due to deficiencies in oxygen transport to tissues and deficiencies in iron-containing compounds, in particular enzymes. The oxidative free radicals formed during ischemic events increase the level of ischemia-modified albumin (IMA) by making chemical changes in the albumin molecule. The state of thiol/disulfide plays a vital role in antioxidant process. We aimed to determine the relationship between native thiol, total thiol, disulfide and IMA in female patients with Iron Deficiency Anemia (IDA).
MATERIALS and METHODS:32 female patients diagnosed with IDA and 24 healthy women were joined in our study. Blood samples were taken for complete blood count (CBC),serum iron,total iron binding capacity (TIBC),ferritin and thiol / disulfide homeostasis tests after fasting for at least 8 hours. IMA Abs. levels were determined by a colorimetric method.Total and native thiols and disulfide were analyzed with a novel spectrophotometric method.
RESULTS:We found lower native thiol (-SH)(378.0±135.6 µmol/L), disulfide (113.5±7.6 µmol/L), and total thiols (-SH + -S-S-) (613.0±125.8 µmol/L) in IDA patients compared to healthy controls (respectively 399.5±158.8, 136.7±63.3, and 707.5±119.23 µmol/L). IMA Abs. levels (0.68±0.11 AU) were higher in IDA patients compared to controls (0.59±0.15 AU). Total thiols levels were positive correlated with both native thiol ($r=0.326$; $p=0.033$) and disulfide ($r=0.511$; $p=0.001$)
CONCLUSIONS:Thiols, disulfide and IMA levels increase with the progression of iron deficiency. IDA decreases the antioxidant capacity of erythrocytes and triggers oxidative stress. Therefore, the hypoxic state and oxidant balance resulting from anemia are important in terms of prognosis of the disease
Keywords: Thiol/disulfide homeostasis, Oxidative stress, Ischemia-modified albumin, Iron Deficiency Anemia

O-048
Effect of hibernation on oxidative equilibrium in ground squirrels

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OBJECTIVES:Animals enter the hibernation by providing the necessary conditions to protect themselves from physiologically adverse seasonal conditions. After hibernation, hibernating animals raise their body temperature to 37 °C within a few minutes and rapidly increase oxygen consumption by 10-20 times. Oxygen scarcity increases the risk of oxidative stress in sensitive tissues in mammalian torpor. The rate of formation of free radicals and their rate of removal are in equilibrium in the organism. The serious imbalance between antioxidant defense mechanism and free radical formation refers to oxidative stress. In our study aimed to determine the level of oxidative stress in

hibernation different time (before hibernation, during and after) in liver, lung, heart and kidney of Spermophilus xanthomorphius, Spermophilus citellus and Spermophilus taurensis living in different ecological zones in Turkey.
MATERIALS and METHODS:Eighteen animals (S. citellus (6), S. taurensis (6) and S. xanthomorphius (6)) were included in our study. Three different condition (at hibernation, aroused and non-hibernation stage) data were compared. They were sacrificed under anesthesia. Glutathione (GSH), reactive nitrogen oxide species (NOx) and malondialdehyde (MDA) levels were measured spectrophotometrically.
RESULTS:MDA levels during the hybridization were significantly higher in all tissues of the three species, while GSH levels were found to be low. After the hybridization, MDA and GSH levels were increased before and during the hibernation.
CONCLUSIONS:Our data show that an impaired balance exists between oxidative stress and antioxidant systems in most organs and tissues during hibernation
Keywords: Hibernation, Oxidative stress, Ground Squirrel

O-049
Cellular protection by Phlomis species in H_2O_2-induced oxidative stress

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OBJECTIVES:Neuroglia-derived chronic inflammation and oxidative stress play central roles in the pathogenesis of neurodegenerative diseases. Thus, increasing evidence indicates that anti-inflammatory activity of plant species and their chemical constituents may protect neurons against various brain disorders. The genus Phlomis is composed of perennial plants in Lamiaceae family which is represented by 46 species from which 30 are endemic in Turkey. The main constituents of Phlomis species are reported to exert pharmacological activities. In this study, we aimed to evaluate the effects of methanol extracts of Phlomis species in H2O2-induced oxidative stress in a cellular model.
MATERIALS and METHODS:At first, antioxidant activities of methanol extract were evaluated by DPPH and ABTS assays. The effects of the extracts on cell viability were determined by using WST-1 assay. Cells were pre-treated with various concentrations (1, 10 and 100 µg/ml) of extracts for 2 h and exposed to H2O2 for 1h.
RESULTS:Similar to the results obtained by antioxidant assays, methanol extract at 10 µg/ml concentration provided 36.40% neuroprotection against H2O2-induced toxicity.
CONCLUSIONS:Our preliminary results indicate that more research is needed to establish the role of endemic plants as a potential source of neuroprotective agents for further therapeutic approaches.
Keywords: oxidative stress, Phlomis species, neuroprotection

O-050
Neuroprotection by optimized system extracts of *Morus nigra* L. Fruits in L-DOPA-induced toxicity

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OBJECTIVES:Morus nigra L. fruits are rich in polyphenols, flavonoids, and anthocyanins responsible for their antioxidant and anti-inflammatory activities. In this study, we aimed to investigate the potential neuroprotection by optimized system extracts of Morus nigra L. fruits in terms of apoptosis related protein expressions.
MATERIALS and METHODS:Extraction of Morus nigra L. fruits was performed with supercritical carbon dioxide, subcritical water and microwave assisted extraction systems as advanced extraction technologies; following conventional methods as orbital shaker and sonification. Obtained extracts in two different concentrations (10 and 100 µg/ml) were evaluated for neuroprotection against L-DOPA-induced cytotoxicity in human neuroblastoma cell lines (SH-SY5Y) by using WST-1 assay. The changes in apoptosis-related proteins (Bax, Bcl-2) were investigated by using Western Blotting technique.
RESULTS:Most of the extracts at indicated concentrations were found to protect cells and regulate Bax and Bcl-2 expression levels in L-DOPA-induced toxicity. The maximum protection was observed following subcritical water extraction system at 100 µg/ml which significantly increased cell survival from 51.68 ± 14.19% (L-DOPA-treated cells) to 161.88 ± 20.12% ($p<0.05$).
CONCLUSIONS:Chosen extracts/fractions were found to be significantly anti-apoptotic. High yields of polyphenols and other antioxidant compounds by advanced extraction techniques may have roles in protecting neuronal cells via regulating Bax and Bcl-2 proteins. Selection of extraction method is a crucial point for plants to achieve therapeutic potential.
Acknowledgements: This study was supported by the TUBITAK (216S839), IKCU BAP and OYP fund supplied by YOK. Novel Fluidic Technologies Laboratory and FABAL (Ege University) are highly appreciated.
Keywords: Neuroprotection, L-DOPA, apoptosis, Morus nigra L. fruits

O-051
Dynamic thiol-disulphide balance and thioredoxin reductase enyzme levels in patients with chronic kidney disease

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OBJECTIVES:We aimed to measure the dynamic thiol-disulfide balance and thioredoxin reductase(TrxR) enzyme levels in patients with chronic kidney disease (CKD) and to investigate their roles in disease pathogenesis by comparing them with systemic oxidative stress and inflammation parameters.
MATERIALS and METHODS:Thirty hemodialyses(HD),30 CKD patients(stage3-5) and 30 controls were included in the study. Fasting blood samples were collected. After centrifugation at 1500g for 10min, serum and plasma samples were portioned and stored at -80°C.IMA levels were determined by albumin cobalt binding test(ACB).Dynamic thiol-disulfide balance was determined by the colorimetric method developed by Erel et al. Tumor necrosis factor(TNF-α) and TrxR levels were determined by ELISA.
RESULTS:We found that native and total thiol levels of CKD and HD patients were significantly lower than that of the control group($p=0.001$ for both). However, disulfide levels were significantly higher in the HD group ($p=0.001$),but there was no significant difference between control and CKD groups ($p=0.547$). A notable negative correlation was found between the native and total thiol levels and IMA(r=-0.628;-0.631),BUN (r=-0.747;-0.747),and creatinine(r=-0.732;-0.721).There was a significant positive correlation between glomerular filtration rate(GFR) and the thiol levels(r=0.835;0.824).TrxR levels were significantly higher in the patient groups compared to the controls($p=0.001$). TNF-α and CRP levels of the patient groups were significantly higher compared to the controls($p=0.001$).
CONCLUSIONS:Colorimetric measurement of dynamic thiol levels can be used in disease monitoring as a marker because it is easily applicable in routine clinical biochemistry laboratories.The dynamic thiol balance may be involved in the pathogenesis of CKD and is associated with disease severity.
This study was supported by Hatay Mustafa Kemal University, Coordinatorship of Scientific Research Projects.
Keywords: Dynamic thiol-disulfide balance, Thioredoxin reductase, Chronic kidney disease, Hemodialysis

O-052
Protective role of lycopene in experimental heart ischemia reperfusion model

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OBJECTIVES:Ischemia refers to the reduction or cessation of blood flow which results in tissue damage and causes insufficient oxygen and nutrition to the tissues. Oxidative stress due to reperfusion after ischemia causes severe functional and structural damage. Free oxygen radicals are responsible for this damage. Lycopene is a pigment of the carotene family, wich is naturally found in vegatables and fruits. To the best of our knowledge, this is the first study, we aimed to investigate the protective role of lycopene in experimental heart ischemia reperfusion (I/R) model.
MATERIALS and METHODS:Male Wistar rats were randomly allocated into three groups (n = 8, each) as control (I/R group), Sham and Lycopene (therapy group) groups. One group received lycopene (50 mg/kg/day as intraperitoneally) for both single dose before surgery (I/R+lycopene group), while the other was treated intraperitoneally with 0.09 % saline as group (0.3 mL/day) (sham group). However, nothing was given to the I/R group. Then; after the venture and surgical procedure applied to the all rats groups, 10 minutes ischemia and 10 minutes reperfusion of the heart was created. At the end of this experimental, activities of catalase (CAT), superoxide dismutase (SOD) and the levels of malondialdehyde (MDA) as oxidative stress biomarkers were measured as spectrophotometric and, also the levels of nitrotyrosine (3-NTx) and nitric oxide (NO) as nitrosative stress biomarkers were measured by ELISA in heart tissues homogenates.
RESULTS:Oxidative/nitrosative stress was confirmed by the significant elevation in MDA, NO, and 3-NTx levels concentrations in I/R group ($p < 0.05$). Also, CAT and SOD activities in I/R group were significantly lower than lycopene and sham groups ($p<0.05$). However, increased CAT and SOD activities and decreased the levels of MDA, NO and 3-NTx were found in lycopene group compared to I/R and sham groups ($p<0.05$).
CONCLUSIONS:We thought that lycopene may play the protective the role against heart I/R damage due to its high antioxidant activity.
Keywords: Lycopene, oxidative/nitrosative stress, heart ischemia-reperfusion

O-055
Oxidative status in degenerated painful ıntervertebral disc samples: variability with respect to duration of symptoms and type of disease

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OBJECTIVES:Degenerated discs and endplate abnormalities is postulated as a possible source of low back pain. Oxidative stress plays an important the role in various human diseases. This is the first study, we aimed to investigate the levels of oxidative stress biomarkers in disc samples of patients with Modic Changes.
MATERIALS and METHODS:Patients (n:15) were separeted as MCI, II, and III types. Of these cases, 3 had complaints for less than 6 months, whereas 3 patients had been suffering from low back pain and leg pain for more than 6 months. Six patients have been diagnosed with subligamentous type and 3 patients had free fragment type of disc degeneration. The activities of catalase (CAT) and superoxide dismutase (SOD), and the levels of malondialdehyde (MDA) in disc samples were determined on spectrophotometer
RESULTS:Oxidative stress was confirmed by the significant elevation MDA levels and decreased of CAT and SOD activities in MCI compared with other MCs ($p<0.05$). The highest CAT and SOD activities were found in patients with MCII compared with the other MCs. However, the levels of MDA showed moderate increase in this group ($p<0.05$). In addition, the levels of oxidative stress biomarkers in patients with MCIII were slightly higher than the other MCs ($p< 0.05$).
CONCLUSIONS:Our findings indicated that oxidative stress in patients with MCI may be aggravated as a result of oxidant/antioxidant imbalance and it may cause formation of the lesion in these patients.
Keywords: : Modic Changes, Disc Samples, Oxidative Stress

O-056
The effect of turmeric on GPER1 and oxidative/nitrosative stress biomarkers in cardiac ischemia reperfusion

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OBJECTIVES:Extreme oxidative stress induced by reperfusion after ischemia causes functional and structural damages. Free oxygen radicals are mainly considered as responsible for the damage. It has been known that there is a connection between cardiovascular diseases and estrogen. Estrogen is effective on estrogen receptors alpha and beta, and also recently a new estrogen receptor depending on G protein has been determined (GPER1). It has been revealed in various researches that turmeric has hypoglycemic, anti-inflammatory, antioxidant and lipid reducing effects. In this first study, it was aimed to investigate the effects of turmeric on GPER1 and oxidative/nitrosative stress parameters in heart ischemia reperfusion injury in rats.
MATERIALS and METHODS:The study was carried out with three groups (treatment, sham and control) of eight rats each. Heart ischemia reperfusion injury was formed experimentally in all rats.Turmeric (50 mg/kg) single dose was given intraperitoneally in the treatment group. Physiological saline (0,09% NaCl, 0,3 mL) single dose was given intraperitoneally in the sham group. No drugs were given in the control group.
RESULTS:Compared to control and treatment groups, antioxidant enzymes (SOD, CAT) decreased and MDA levels increased in the ischemia reperfusion group ($p<0,05$). On the other hand, the levels of antioxidant enzymes in the treatment group approached the control group and MDA levels decreased ($p<0,05$).
CONCLUSIONS:As a result of this study, it was determined that turmeric has a protective role against heart ischemia reperfusion injury.
Keywords: Turmeric, cardiac ischemia reperfusion injury

O-057
The impact of acupuncture treatment on dynamic thiol–disulphide homeostasis and ischemia-modified albumin levels to assess

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OBJECTIVES:The aim of this study was to investigate the effect of acupuncture on dynamic thiol–disulphide homeostasis and ischemia-modified albumin (IMA) levels as a novel oxidative stress parameter in migraine patients.
MATERIALS and METHODS:The acupuncture treatment consists of 5 sessions with 2 sessions per week. Blood samples have been collected before performing acupuncture, after the 1st and 5th session of the acupuncture. And for the control group blood samples were collected only once. In this study, the dynamic thiol–disulphide homeostasis and IMA levels in the serum samples of migraine patients and healthy individuals was determined using an automated method newly developed by Erel et al.
RESULTS:There were statistically significant differencies %SS (Disulphide) / total thiol levels patient with pre and post acupuncture groups compared with control group ($P<0.05$). However there was no relationship %SS (Disulphide) / total thiol levels patient with post acupuncture groups compared with pre acupuncture groups ($P >0.05$). The average %SS (Disulphide) / total thiol levels

was found to be 9,00±3,27 mmol/lt in the patient group and 6,98±2,62 mmol/lt in the control group. The total %SS (Disulphide) / total thiol levels of patient group were found to be higher than the control group but not statistically. Thiol disulfide balance and IMA levels, which are oxidative stress markers, were increased in migraine patients compared to the control group and this was statistically significant ($p < 0.05$). We found that acupuncture treatment caused some decrease in thiol disulfide balance but these results were not statistically significant. Ischemia-modified albumin (IMA) were not correlated with attack frequency, pain intensity, or migraine type. Only 5 sessions could be given to these patients. It is possible that if the number of sessions is increased, a meaningful result can be achieved.
CONCLUSIONS:This study evaluated dynamic thiol–disulphide homeostasis and IMA levels in the serum of patients diagnosed with migraine using a novel automated colorimetric method. Because oxidative stress plays an important role in the pathogenesis of many diseases, thiol chemistry has been recognized as increasingly important. We think the effect of acupuncture on dynamic thiol–disulphide homeostasis and IMA in migraine patients has revealed that further animal and human studies are necessary.
Keywords: Migraine; Acupuncture; Complementary Therapies, Oxidative stress;

O-058
Effect of N-acetylcysteine on cisplatin induced apoptosis in rat kidney

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OBJECTIVES:Cisplatin is one of the most potent and widely used chemotherapeutic agents for treatment of a wide variety of solid tumors in clinic. However, due to various side effects such as nephrotoxicity, its efficiency and therapeutic application are limited. Regarding to reduce its side effects, combination therapies of cisplatin with other drugs have been highly considered to reduce toxicity. N-acetylcysteine (NAC), the N-acetyl derivative of the natural amino acid L-cysteine, is a well known antioxidant and anti-inflammatory agent. In the current study it was aimed to investigate the effects of NAC on cisplatin induced apoptosis in rat kidney.
MATERIALS and METHODS:Twenty four male Wistar rats were separated into 4 equal groups: Control, NAC-250, CP (cisplatin), CP+NAC. Rats in the experimental groups were treated with a single dose of cisplatin intraperitoneally (ip) (10 mg/kg) and NAC (ip, 250 mg/kg) for 3 consecutive days. At the end of the experiment, nephrotoxicity was confirmed by blood urea nitrogen and creatinine levels and the apoptotic changes were demonstrated by TdT-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) and caspase-3 levels in rat kidneys.
RESULTS:The number of TUNEL-positive cells and caspase-3 levels were significantly increased by cisplatin at day 3 after its injection. Treating the rats with NAC significantly decreased TUNEL-positive cells and caspase-3 levels.
CONCLUSIONS:These data suggest that apoptotic cell death are involved, at least in part, in the pathogenesis of cisplatin induced nephrotoxicity, and inhibition of apoptosis appears to play a central role in the beneficial effects of NAC.
Keywords: Cisplatin; Apoptosis; Caspase-3; N-acetylcysteine; Rat

O-060
Thiol-disulfide homeostasis in diabetic microvascular complications

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OBJECTIVES:Retinopathy, neuropathy and nephropathy are microvascular complications of diabetes mellitus. In this study, the role of thiol / disulfide was investigated in the development of diabetic microvascular complications.
MATERIALS and METHODS:Individuals (n=266) were divided into five groups; Group 1; who have diabetes without any complications for at least 10 years, group 2; diabetic nephropathy, Group 3; diabetic neuropathy, 4; diabetic retinopathy. The 5th group consisted of 50 healthy individuals as the control group. Thiol, disulfide, ferroxidase and ischemia modified albümin (IMA) levels were measured.in the serum.
RESULTS:Nativ thiol, total thiol and native thiol / total thiol were found lower in the retinopathy group than the group with at least 10 years diabetes without any complication, the neuropathy group and the control group ($p < 0.001$). Disulfide / native thiol and disulfide / total thiol levels were found to be higher in the retinopathy group than all the other groups, also the level of disulfide was higher than the control group and neuropathy group ($p < 0.001$). Ischemia-modified albumin level was found to be higher in the neuropathy and retinopathy groups than all the other groups ($p < 0.001$). Ferroxidase level was found to be lower in the neuropathy and retinopathy groups than the nephropathy group.
CONCLUSIONS:The disruption of thiol disulphide homeostasis favor of disulfide may play a role in the formation of diabetic retinopathy. Also, increased IMA and decreased feroxidase levels may play a role in the development of diabetic retinopathy and neuropathy.
Keywords: Diabetes mellitus, microvascular complications, thiol- disulfide, ischemia modified albumin, ferroxidase

O-061

Biological variation in clinical practice: Bridge between laboratorians and clinicians

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OBJECTIVES:Laboratory-related errors are important part of the medical errors. Clinicians usually consider laboratory test results as absolute values. However, laboratory tests have pre-analytical, analytical and biological variations (BV). The aim of this study was to search how the knowledge and developments produced in the laboratory medicine were used by clinicians for the benefit of the patients. For this purpose, we selected BV as a model and investigated how clinicians use BV data to interpret test results.
MATERIALS and METHODS:A survey comprising 399 clinicians was conducted to evaluate knowledge regarding the BV in Turkey. We prepared a questionnaire consisting 9 questions. Five questions were open ended and 2 of the open ended questions were case based. A scoring system A to D (A indicates correct interpretation and D indicates clinician has no knowledge on variations) were used to evaluate open ended questions.
RESULTS:Clinicians (46%) used combination of the reference interval, clinical evaluation and literature data to interpret test results. In open-ended questions 83% of clinicians scored C or D. None of the clinicians were using the reference change value in monitoring test results. Clinicians did not read an article about BV (88.3%) and they were not trained about BV (82%).
CONCLUSIONS:Clinicians are not adequately familiar with the new developments in laboratory medicine and do not use BV data to interpret tests results. Effective communication/collaboration between laboratorians and clinicians will enable clinicians to interpret laboratory tests accurately and use BV data more efficiently.
Keywords: Biological Variation, Interpretation of test results, Laboratory-Clinic Interaction

O-062

Using the model of quality indicators: A pilot study

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OBJECTIVES:Continuous monitoring of laboratory performances is a key activity in identifying errors and promoting improvement in Laboratory Medicine. Since 2008, The Working Group "Laboratory errors and Patient safety (WG-LEPS) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has designed a Model of Quality Indicators (MQI) and implemented an informative platform to collect QI from laboratories worldwide. Data collected are processed, and a report describing the laboratory results compared with those of other participating laboratories is periodically issued. A working group is established in Turkish Biochemical Society to use of these quality indicators and to contribute harmonisation studies. The aim of the study was to determine usage of MQI on practical area.
MATERIALS and METHODS:Bağcılar Training and Research Hospital Laboratory has been chosen to enter their own data for MQI project. After registered to the project web site, data entering has been started since may 2017. There were nine selected QIs, four QI were about pre-analytical phase and five QIs post-analytical phase.
RESULTS:WG-LEPS publishes performance reports for each indicator as annualy. According to these published reports our laboratory data which are entered to the system meet high and medium performance criteria.
CONCLUSIONS:There are lot of QIs that are offered. One of the biggest challenges is the difficulty in understanding some indicators. Quality indicators should be translated to all languages and there should be more detailed explanation and calculation methods. Due to difficulties in obtaining data from laboratory information system, a common midware is needed.
Keywords: quality indicators, wg-leps, laboratory errors, patient safety

O-063

National guidelines for the preparation, distribution and testing of purified water for clinical laboratories

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OBJECTIVES:The aim was to prepare a national guide for the preparation, distribution, and testing of purified laboratory water in clinical laboratories in Turkey.
MATERIALS and METHODS:Laboratory Water Guide Working Committee was established by Turkish Biochemical Society in 2017. It contained seven clinical biochemists. After appointing director, the treasurer, technical experts, method scientists and editors were defined. The needs for the country were determined. The strategic plan was made. The partners were identified. After that, work management was established; literature and resources were reviewed. The study was started. Total twelve meetings were performed in different cities. The study completed in one and a half year. After evaluation and approval, it was reported and the guide book was published in June 2019.
RESULTS:This national guideline defines the Laboratory Water Purification System (LWPS). It covers the subjects of laboratory water types and quality characteristics, water pollutants, laboratory water purification methods, storage and distribution, validation and monitoring of the water system and sampling and testing of laboratory water. Laboratory water types and quality characteristics were described based on CLSI. The guide defines minimum laboratory water quality standards to be considered and the processes to improve water quality in clinical laboratories. It also includes the problems that may be encountered

during design, and the solutions. LWPS should be operated under control and maintained. Thus, it ensures operational stability and meets water quality control standards.
CONCLUSIONS:This guide is national, and provides to clinical biochemists the information about operation, storage, monitoring and use of laboratory water system.
Keywords: Laboratory Water Purification System, Clinical Laboratories, Turkish Biochemical Society, Working Committee

O-064
Evaluation of CKD-EPI Pakistan equation for estimated glomerular filtration rate (EGFR) in Pakistan

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OBJECTIVES:To evaluate the results of 24-hour urinary creatinine clearance (CrCl) with estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), CKD-EPI Pakistan (CKD-EPI Pak), Cockcroft Gault (CG) and 4-variable Modification of Diet in Renal Disease (MDRD) equations.
MATERIALS and METHODS:A descriptive, cross-sectional study was conducted at the section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, The Aga Khan University, Karachi. Laboratory data of subjects' ≥18 - < 70 years ordering 24-hour urinary CrCl was retrieved. Statistical comparison of eGFR using CKD-EPI, CKD-EPI Pak, CG and MDRD with the timed urine collection CrCl was done using regression analysis.
RESULTS:The mean age of the group (n=670) was 51.3 ±15.4 years with a median of 53 (IQR:22.3) years, 55.7% being males. Median BMI of males and females was 26.98 kg/m2 (IQR: 7.09) and 26.16 kg/m2 (IQR: 6.97), respectively. Mean GFR using 24-hour creatinine clearance was 57.1 ±35.9 ml/min/1.73m2 with a median of 51 ml/min/1.73m2. Urinary creatinine clearance showed strong correlation with CG, MDRD, CKD-EPI and CKD-EPI Pak, showing r=0.78, r=0.79, r=0.82, and r=0.82, respectively. Sensitivity was highest for the CKD-EPI Pakistan (84.7%). Similarly, CKD-EPI Pakistan equation showed the highest agreement (88.7%) with CrCl compared to the other formulae.
CONCLUSIONS:The CKD-EPI Pak equation is more accurate and precise than the CG, CKD-EPI and MDRD in estimating GFR in Pakistani population. The results of this study support automated reporting of eGFR using CKD-EPI Pak equation in laboratories across Pakistan.
Keywords: estimated glomerular filtration rate, equations, Pakistan, adults

O-065
The local technical validation of Barricor™ tube that uses a mechanical separator

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OBJECTIVES:Unsuitable samples are common problems for laboratories. The blood collection tubes need to be validated and verified prior to routine laboratory administration in order to reduce this problem. In this study, we aimed at comparing the technical qualifications of routinely used BD Serum Separator II/SST II™ tubes with BD Barricor™ LH tubes for local technical validation.
MATERIALS and METHODS:150 volunteers were enrolled in the study. Samples were collected in two tubes by a single phlebotomist. 12 quality indicators were evaluated. The difference (%) was calculated with the formula proposed by EFLM. In case of any difference of less than 1% for indicators, the evaluated tube was considered adequate.
RESULTS:Indicators, such as tubes with physical defects of manufacturing, with no vacuum or that fail to create vacuum, not properly fitting into the blood collection device, under filling (10%), cracked tubes or tubes with leaking from the cap before/after centrifugation, blood contamination of collection device, haemolysed specimens, incorrect positioning of separator after centrifugation, tubes including fibrin strand/mass in sample after centrifugation, red blood cell adhesion to interior tube walls after centrifugation were found adequate in Barricor™ tubes. White particulate matter (WPM) was observed in 24.6% of Barricor™. Therefore, the last indicator, tubes including gel/foreign material/WPM in sample after centrifugation, was found inadequate in Barricor™.
CONCLUSIONS:It was thought that WPM with 24.6% presence would not cause any interference in a properly filled tube. Thus, Barricor™ was found to be technically adequate. Technical validation studies should be encouraged in terms of total quality management.
Keywords: Phlebotomy; blood collection devices; preanalytical errors; technical validation; quality management

O-066
The utility of preanalytical quality indicators: A Turkish survey study

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OBJECTIVES:The utility of quality indicators(QIs) to monitor the total testing process of laboratories is able to improve the quality of services and enhance patient safety. The present study set out to investigate the harmony between the preanalytical QIs within the context of Model of Quality Indicators (MQI) determined by IFCC Working Group on Laboratory Errors and Patient Safety (WG-LEPS) and the QIs used by Turkish medical biochemistry laboratories. The other purpose of this investigation is to assess the usability of IFCC preanalytical QIs considering the conditions of Turkey.
MATERIALS and METHODS:A survey consisting of 9 questions prepared by Turkish Biochemical Society Working Group of Laboratory Errors and Patient Safety was applied to 81 laboratories via Survey Monkey.
RESULTS:According to survey results, 91 percent of participant laboratories used QIs proposed by Ministry of Health Quality Standards in Health. While some QIs within the context of MQI were utilized by over 80 percent of laboratories, some of other QIs were used by under 10 percent of laboratories.
CONCLUSIONS:The majority of the laboratories utilized QIs determined by Ministry of Health Quality Standards in Health and Ministry of Health On-site Assessment Guide. These standards were found to be partially compatible with IFCC WG-LEPS QIs. The inability of the health information system (HIS) limits the usage of QIs proposed by WG-LEPS. Education of medicial biochemistry specialists and other healthcare personnel and improvement of HIS are crucial for the QIs usage. Definitons of QIs should be more plain and understandable.
Keywords: laboratory errors, preanalytic phase, patient safety, quality indicators

O-067
A web-based application for management of quality control data

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OBJECTIVES:In medical laboratories, internal quality control (IQC) is the main quality control measure to assess the analytical phase. The main role of IQC is to detect errors in the analytical mom to assure patent safety. Regulations also obligate laboratories to conduct an IQC scheme. The main gap in IQC is the evaluation of results and existing laboratory information systems (LISs) mainly lack state of the art evaluation methods for QC data. Although there are some advanced software solutions for management of QC data, license costs can be a burden for laboratories. The aim of this study is to develop an open source web application for evaluation of quality control results.
MATERIALS and METHODS:In this study, a web-based QC data management

software was developed by using R programming language. Shiny tools were used for user-friendly interface. For sigma metrics calculation, different recommendations for quality requirements were utilized. For this software, QC data can be extracted from either LIS or middleware.
RESULTS:General features of this software are evaluation with given target values, revision of target values from accumulated data, evaluation with multi rules, calculation of bias from EQA results, calculation of total error and sigma metrics. This software can be applicable for daily IQC monitoring, periodic reporting and analytics.
CONCLUSIONS:This web-based software provides a more accessible way to correct QC practices for clinical laboratories.
Keywords: Quality control, internal quality control, data management software, open source

O-068
Quality control application for CBC parameters by 'Average of Normals' method

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OBJECTIVES:Application of internal quality control (IQC) with 'average of normals (AON) method for complete blood count (CBC) parameters and to validate AON method with biased data.
MATERIALS and METHODS:For this study, CBC results in a Sysmex XN-series instrument in our hospital's hematology laboratory in July 2019 were evaluated. AON method was applied using Sysmex XbarM program. XbarM is based on calculation of weighted moving averages derived from Bull's algorithm. Using XbarM 'target/limit' function, laboratory-specific target values were calculated automatically. When target and % values specified for control limits were entered into XbarM, weighted averages were calculated for each batch of results. A simulation study was performed with the biases of -80%,-50%,-20%,-10%,-5%,-2%,2%,5%,10%,20%,50%,100% on the existing patient data. Using Excel, based on formula used by XbarM, new moving averages were calculated and evaluated for each bias point. Averages were taken every 50 patients. Target values for our laboratory were calculated automatically by monitoring patient results, control limits were determined according to % values recommended by Sysmex.
RESULTS:During follow-up, instrument had 2-8 AON points daily. There was no value exceeding control limits and this was consistent with conventional IQC. When biases were added, AON method could not detect 2% bias in general, but 5% and above could be detected in early period.
CONCLUSIONS:AON method is cost-effective and complementary to conventional IQC programs especially to detect systematic errors.
Keywords: Average of Normals, Moving Averages, Quality Control, CBC

O-069
Evaluation of the most common rejection reasons in the preanalytical process at our laboratory using six sigma analysis

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OBJECTIVES:Based on statistical calculations, "Six Sigma Level" provides information about process performance In our study, we aimed to determine the most common reason for rejection of the samples rejected in the preanalytical period and to evaluate the effect of corrective and preventive actions to reduce this rate by using six sigma level analysis.
MATERIALS and METHODS:The quality studies which were conducted in 2019 at our laboratory showed that the most common reason for rejection in preanalytical period was inadequate sample collection in sedimentation test. Educational programme was organized for the related units to correct this ratio. After the educational training, sedimentation test rejection rates were re-examined and evaluated.
RESULTS:When the distribution of rejection reasons was examined, it was found that the highest rate was inadequate sample collection the rate was 28.92%.the most frequently rejected test was ESR test with a rate of 55%. Educational program was provided to related units. One month sigma values were calculated before and after educational training. in June 2019(pre-education) sedimentation test total number was: 7455, rejected sample number: 418, sigma level was 3.1. in July 2019 (after the educational program has been applied) total number of samples was 9534, rejected sample number: 335 sigma level was determined as 3.4.
CONCLUSIONS:The analysis of the main cause of preanalytical problems in the laboratory and the initiation of corrective and preventive actions increase the quality of the laboratory. We believe that sigma values will improve significantly by increasing the frequency of the educational training programs.
Keywords: Preanalytic phase, rejection rates

O-071
Analytical performance of Cobas 6500 for predicting urinary tract infection

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OBJECTIVES:Urinary tract infection (UTI) is the most common disease in the community. Urinalysis is the most requested screening test in patients with symptoms possible UTI. We aimed to compare the dipstick and sediment analysis results of fully automated Cobas 6500 urine analyser with gold standard urine culture results.
MATERIALS and METHODS:Data of 571 patients with order for urine dipstick test, urine sediment analysis and urine culture between March and November 2018 were evaluated retrospectively. Sensitivity, specificity, positive and negative predictive values and ROC curve analysis was performed for leukocyte esterase (LE) and white blood cells (WBC).
RESULTS:349 of 571 patients had positive urine culture results. The sensitivity of dipstick leukocyte esterase was found to be 73.35%, whereas the specificity was 61.71%. Positive and negative predictive values were 75.07% and 59.56, respectively. WBCs showed 70.77% sensitivity with 65.31% specificity with positive and negative predictive values of 76.23% and 58.7, respectively. The area under the curve (AUC) for LE and WBC were 0.707(0.668-0.744) and 0.753(0.716-0.788).
CONCLUSIONS:Leukocyte esterase in urine dipstick test and microscopic WBC tests had comparable results in predicting UTI. Clinical decisions based on dipstick urine and sediment analysis could be both time and cost effective and may reduce the need for the conventional urine culture.
Keywords: Cobas 6500, leukocyte esterase, urine culture, WBC

O-072
A comparison of Sysmex UF-5000 flow cytometer and Fuchs-Rosenthal Chamber in urine sediment analysis

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OBJECTIVES:Urine analysis is a basic test in the clinical laboratory. Urine sediment analysis is a part of urine analysis that gives laboratory professionals valuable information. Since manual examination is the gold standard for analysis it is time consuming and work-intensive procedure. In this study we aimed to compare the performance of Sysmex UF-5000 flow cytometer with the manual Fuchs-Rosenthal chamber in terms of urine sediment analysis.
MATERIALS and METHODS:A total of 127 fresh urine samples from outpatient clinics are analyzed. We used Sysmex UF-5000 flourescence flow cytometer for urine analysis and Fuchs-Rosenthal chamber for urine sediment analysis.We compared two methods by using Passing-Bablok regression analysis, Pearson correlation coefficient (r) and Bland-Altman bias plot.Statistical analysis was performed using Analyse-it software version 3.80 (Analyse-it Software,Ltd., Leeds, UK), Microsoft Excel 2010, and CLSI Statis-Pro software version 3.0.
RESULTS:A good correlation was observed between manual and automated white blood cell (WBC) counts in all urine samples. (r = 0.988; y = 1,162x + 0,489; n = 127). UF-5000 demonstrated a significant proportional overestimation with Passing–Bablok regression (95% CI slope: 1,110 to 1,226). For red blood

cell (RBC) counts,correlation between UF-5000 and the counting chamber was observed in all samples (r = 0,966; y = 1,1x + 0,75).
CONCLUSIONS:This study showed us that urine analysis with flow cytometers is a very promising area and with automation getting more commonly used in clinical laboratories in the world,it is likely to replace the manual microscopy and thus reduce the workload and also time and energy needed in laboratories.
Keywords: Urine analysis, flow cytometer, Fuchs-Rosenthal chamber

O-073
Determination of serum carbamazepine by tandem mass spectrometry

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OBJECTIVES:Carbamazepine is a first-line drug for the treatment of different forms of epilepsy. Therapeutic range of carbamazepine in plasma is 5 to 10 µg/ml; more specifically, 7.4 µg/ml for adults and 8.2 µg/ml for children. Carbamazepine plasma level is directly correlated with dose, therapeutic effect and side effects. Carbamazepine plasma level is affected by several factors. It is altered by age and pregnancy status including several other factors. Individualization of drug dose with the help of plasma level detection is a must in case of carbamazepine therapy. In this study, our aim was to develop a LC-MS/MS method for the measurement of carbamazepine.
MATERIALS and METHODS:100 µL of the internal standard (gliclazide solution) on a standard solution or sample of 250 µL was vortexed for 30 s by adding 500 µL of acetonitrile included %0.1 formic acid, followed by centrifugation at 12 000 rpm for 10 min. The supernatants were taken into glass tubes and evaporated with nitrogen gas. The residue was dissolved in 200 µL of in the mixture of acetonitrile:water (50:50;%v:v) then enjected into LC-MS/MS system.
RESULTS:The calibration curve for carbamazepine was established at a range of 0.15 to 80 µg/ml. Detection limit and quantitation limit for carbamazepine; 0.15 µg/ml and 0.3 µg/ml, respectively. The retention time was determined 1.62.Total run time was 5 minutes.
CONCLUSIONS:We can conclude that the developed method can be useful for clinical studies and routine therapeutic drug monitoring with the desired precision and accuracy.
Keywords: Tandem mass spectrometry, drug monitoring, carbamazepine

O-074
3D placental barrier models: A novel cryogel based method

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OBJECTIVES:Cryogels are formed below the freezing point of the solvent. Their advantages are their inherent interconnected three dimentional (3D) macroporous structure and utilization as a scaffold in tissue engineering. At the same time, cryogels supply biocompatible property, ECM support and will represent in vivo better. Poly (2-hydroxyethyl methacrylate-glycidyl metacrylate) [p(HEMA-GMA)] cryogels are super-macroporous (10-100 µm), hydrophilic cryogels. They provide non-specific protein interactions at the minimum level, are mechanically and chemically stable and they are resistant to microbial and enzymatic reactions. In tissue engineering studies, fibronectin potentially increases the cell adherence and folic acid improves the viability of cells.
MATERIALS and METHODS:In this study, galactose containing p(HEMA-GMA)/gelatin cryogels were synthesized and then fibronectin or folic acid was attached on the surface. The proliferative and adhesive effects were investigated by seeding BeWo human placental choriocarcinoma cell line on the cryogels. Cryogels was characterized by swelling test and scanning electron microscopy (SEM). The viability of the BeWo cells on different crogels were investigated by Alamar blue assay 48 h after incubation.
RESULTS:BeWo cells and cryogels have been observed to interact well and cells were proliferated successfully. Among the cryogel groups, p(HEMA-GMA)/gelatin-folic acid group had the highest cell viability. Cell viability was lower in galactose-bound cryogel groups than in galactose-free groups.
CONCLUSIONS:This novel placenta model reflects the in vivo more precisely and can be used as a model in transport of xenobiotics and their metabolites such as newly developed and mandatory drugs used in pregnancy and also for cosmetics, cleaning products, food additives, nanoparticules.
Keywords: placenta, cryogel, scaffold, cell viability

O-075
Antioxidant effects of flavonoid neoeriocitrin on streptozotocin-induced INS-1E cell diabetic model

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OBJECTIVES:Diabetes mellitus is a common metabolic disease, and its prevalance has been increasing globally. Numerous studies have revealed that generation of reactive oxygen species play a crucial role in diabetes. Moreover increasing in DNA damage also is a cause of experimental diabetes. Flavonoids have been promising therapy especially for preventing diabetes mellitus. Neoeriocitrin is a flavonoid which is found in citrus species and it could be a promising agent in preventing β-cells against diabetes. Our aim in this study is to reveal the antioxidant effects of neoeriocitrin against STZ-induced diabetes model in INS-1E cells.
MATERIALS and METHODS:INS-1E cells (rat insulinoma cell line) were pre-incubated with neoeriocitrin at various concentrations (0, 0.25, 0.5, 1 µM) for 21 hours then 5 mM streptozotocin (STZ) were added into cells and incubated for 3 hours. STZ, which is a DNA alkylating agent, was used for inducing diabetes in INS-1E cells. Antioxidant ezymes such as superoxide dismutase (SOD) and catalase (CAT) were measured in cell lysates by spectrophometrically. Additionally, oxidized guanine species as a marker of DNA/RNA oxidative damage was measured spectrophotometrically.
RESULTS:STZ-induced INS1-E cells showed elevated SOD activity and decreases in CAT activity. Antioxidant status of the cells changed by neoeriocitrin treatment. DNA/RNA oxidative damage increased by STZ treatment, and neoeriocitrin caused changes in oxidized guanine species.
CONCLUSIONS:Biologically flavonoids have potential to reduce free radicals and risk of diabetes. Neoeriocitrin which has an antioxidant activity is a promising agent, however further studies are needed to exert mechanism of action against reactive oxigen species in INS-1E cells.This research was financially supported by Hacettepe University Scientific Projects Coordination Unit (Project No: FBA-2018-16746).
Keywords: Neoeriocitrin, flavonoid, diabetes, INS-1E cells, oxidative stress

O-076
***In vitro* investigation of *Argiope bruennichi* derived spider silk materials**

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OBJECTIVES:Spider silks' exceptional chemical and mechanical properties lead to extensive researches in both industry and medicine. The purpose of this study is to investigate the effects of a novel designed biological material, the dragline silk of *Argiope bruennichi*, in surgical applications. As a first step towards this purpose, *in vitro* cytotoxicity assays were performed.
MATERIALS and METHODS:*A. bruennichi* specimens were collected from the Eastern parts of Black Sea region. Special made dragline silking system was used to collect the spider silk material for filament preparation. Nanoscopic structure analyses of the silk filaments were done by SAXS (Small Angle X-ray Scattering) and WAXS (Wide Angle X-ray Scattering) methods. Ab-initio 3D nanoscale morphologies were also obtained by using SAXS data.

Cytotoxic potentials of spider silk based suture materials and their nanocomposite/biopolymer coated (film/filament) forms were investigated in L929 fibroblast cells by MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) and LDH (Lactate dehydrogenase) assays.
RESULTS:SAXS analyses indicate that biopolymer coating causes the most stable 3D nanoglobular aggregations while the nanocomposite coating is convenient to keep the natural nanostructures of the filaments. The results of *in vitro* studies showed that dragline silk of *A. bruennichi* and nanopowdered levan coated films of *A. bruennichi* silk filaments did not show any cytotoxic effect on L929 cells.
CONCLUSIONS:According to the results of *in vitro* studies, *Argiope bruennichi* silk has a potential usage area in surgical applications. Further in vivo studies will be proceeded to investigate the effects of these silk materials on wound healing process in a dorsal skin flap model on rats.
Keywords: spider silk, levan, nanopowder, MTT, LDH

O-077
MicroRNAs in patients with type 2 diabetic nephropathy

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OBJECTIVES:To evaluate the associations of the diabetic nephropathy (DN) with 10 miRNAs that were found to be related to diabetes in human (miR-21a-3p, miR-29a-3p, miR-29b-3p, miR-29c-3p, miR-126-3p, miR-192-5p and miR-320c) and animal (miR-129-1-3p, miR-137, miR-212-3p) studies.
MATERIALS and METHODS:Plasma miRNAs were analyzed by RT-PCR and correlations with the eGFRs were evaluated in 50 healthy controls (male: n=24; age:55±11; female: n=26; age:54±13) and 100 type 2 diabetics (T2DM) (male: n=46; age:60±11; female: n=54; age:56±11). Diabetics were divided into normoalbuminuric (NAlb, n=51), microalbuminuric (MicAlb, n=25) and macroalbuminuric (MacAlb, n=24) groups. Forty-nine diabetics were diagnosed as DN.
RESULTS:miR-21 were detected in approximately half of all groups, and found significant ($p<0.05$) decreases in the T2DM, DN and MacAlb than those in the controls (T2DM: 5-fold, DN: 7, MacAlb: 7). The decrease in miR-192 that were detected in all groups was found significant ($p<0.05$) (T2DM: 2-fold, DN:2.4). The eGFR-based on cystatin C showed positive correlations ($p<0.05$) with miR-21, miR-192 and miR-126 (r=0,262, r=0,203, and r=0,417, respectively). miR-21 and miR-29c were correlated ($p<0.05$) with MDRD eGFR (r=0,243 and r=0,188, respectively). The correlation of CKD-EPI-creatinine with miRNA-192 was significant (r=0,185, p=0,023). miR-21, miR-192, miR-29c and miR-320 were negatively correlated ($p<0.05$) with microalbuminuria (r=-0,323, r=-0,267, r=-0,173 and r=-0,172, respectively). miR-21 and miR-192 were found to be significant in distinguishing the DN from healthy subjects (AUC=0,726, p=0,0001 and AUC=0,717, p=0,0001, respectively).
CONCLUSIONS:miR-21 and miR-192 could be related to DN. More research is needed for the association of miR-29 family, miR-126, miR-212 and miR-320c with DN.
Keywords: Diabetic nephropathy, microRNA, miR-21, miR-192

O-078
Differentiation of osteopetrotic IPSC to osteoclasts: Comparison of osteopetrotic&healthy osteoclast

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OBJECTIVES:Malign infantile osteopetrosis is a lethal, rare genetic bone-disease characterized by dysfunctional-osteoclasts. The only available treatment for this disease is hematopoietic stem cell transplantation.
MATERIALS and METHODS:The main-purpose of this study was to evaluate the osteoclast-dysfunction using osteopetrotic-İPSC-derived-osteoclasts. Firstly,iPSC-lines from both TCIRG1-mutation positive osteopetrosis-patients and a healthy-donor were differentiated into hematopoietic stem cells-(iHSCs), which were characterized with colony-forming-capacity and CD34+CD45+surface-markers by flow-cytometry. Immunophenotyping was evaluated for myeloid-immnunuphenotyping. Then iHSCs were differentiated into myeloid-progenitors followed by osteoclast differentiation using M-CSF,RANK-L. Immunophenot yping,immunofluorescence(IF)-staining,Scanning-electron-microscope(SEM) analysis, and gene-expression profile related to functionality and maturation of iPSCs-derived-osteoclasts were performed.
RESULTS:All iPSC-lines were differentiated succesfully into iHSCs. Patient-iHSCs were showed to have three-times more CFU-M potential comparing to donor, and BFU-E potential was observed only donor-iHSCs. Osteopetrotic-iPSCs-derived-osteoclasts were stained weakly-positive with Cathepsin-K,and Rhodamine-phalloidin comparing to the control. Osteopetrotic-iPSC-derived-osteoclasts were positive for TRAP, imagined as giant-multinucleated-cells, and over 95% of the cells were CD14+CD16+,and CD18+CD51/61+..SEM-images showed that there was a difference between the size of podosomes of patient-and donor-osteoclasts. Osteopetrotic-osteoclasts showed a delayed expression of related functional-genes compared to controls. At the end of differentiation osteopetrotic-osteoclasts showed significantly reduced expression of Cathepsin-K, Calcitonin-R, and NFATC1 genes comparing to controls. All results indicate that both donor-and osteopetrotic-İPSCs were differentiated into osteoclasts, but osteopetrotic-osteoclasts showed different gene and protein expression patterns, and size of podosomes indicating a disease-specific functional impairment. Functionality analyses are ongoing.
CONCLUSIONS:The results of our study might help to increase our knowledge about normal-and osteopetrotic-osteoclastogenesis, but needs to be supported with more detailed functionality-analyses.
Keywords: Osteopetrosis, Osteoclasts, TCIRG1, Induced Pluripotent Stem Cells

O-079
Monodisperse-porous metal oxide microspheres with peroxidase/oxidase mimetic activity as a new tool for biomolecule determination

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OBJECTIVES:Peroxidase-oxidase mimetic activity materials are important for developing new commercial assays.
MATERIALS and METHODS:A new staged-shape templated hydrolysis and condensation protocol was developed for the synthesis of monodisperse-porous metal oxide microspheres.The magnetic forms were synthesized with the accessible magnetic hematite (Fe_3O_4) nanoparticles tightly immobilized within the porous interiors of microspheres.Magnetic SiO_2 microspheres and plain CeO_2 microspheres exhibited peroxidase mimetic activity while oxidase-mimetic activity was obtained with the plain MnO_2 microspheres.
RESULTS:3,3',5,5'-tetramethylbenzidine (TMB) and o-phenylenediamine (OPDA) were used as the synthetic substrates providing products via oxidase/peroxidase-like activities of corresponding microspheres, whose formation kinetics were followed by UV-Vis spectroscopy or fluorescence spectroscopy. The interaction of plain and ligand-attached forms of porous metal oxide microspheres with the biomolecules resulted in a change in the peroxidase-oxidase mimetic activity which was proportional to the biomolecule concentration in the interaction medium, due to the selective adsorption of biomolecule onto the surface of microspheres.
CONCLUSIONS:The concentration of ascorbic acid was determined via oxidase-like activity of plain, porous MnO_2 microspheres, using both colorimetric and fluorometric protocols. The ligand attached forms of plain SiO_2 microspheres were evaluated for developing a colorimetric assay for the determination of histidine tagged protein concentration.We believe that the methodology

exemplified here will be effectively used for developing new commercial assays for the estimation of concentrations of various agents in biological samples.
The research was supported by Hacettepe University Scientific Research Projects Coordination Unit contract numbered as FBA-2019-17337, entitled '' Development of a peroxidase-microzyme-based biosensor for protein determination''. The financial support provided is gratefully acknowledged.
Keywords: Peroxidase activity, Kit fabrication, DNA, Protein, Ascorbic acid.

O-080
Evaluation of analytical process performance by Six Sigma methods in laboratories

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OBJECTIVES:Clinical laboratories are responsible for producing reliable, reproducible and accurate test results.Six sigma is a quality managemen strategy that enables evaluation of processes, identification and improvement of defects. In this context, the aim of our study is to evaluate the analytical process performance of routine tests in our laboratory with six sigma method.
MATERIALS and METHODS:Internal quality control(IQC) data of tests were obtained retrospectively. Mean, standard deviation and coefficient of variation values of IQC were calculated. Process sigma values were calculated using the formula "%Allowable Total Error (TEa)- Bias%/CV% ". TEA values were determined according to CLIA 88. Sigma ≤3 was considered as low quality, sigma between 3 and 6 was considered as good quality and Sigma≥ 6 was considered as world class quality.
RESULTS:In the tests we evaluated, all of the process sigma were>3.The sigma levels of IQC1 for albumin, creatinine, LDL, urea, chloride,total cholesterol,HDL and sodium and IQC2 sigma for albumin, urea, UIBC, chloride, creatinine, potassium, sodium, direct bilirubin tests were between 3-6.The sigma of IQC1 for ALP, ALT, AST, CK, CKMB, ıron, uıbc, phosphorus, GGT, glucose, calcium,LDH, Mg,potassium, total protein, triglyceride, uric acid, amylase, lipase, direct bilirubin, total bilirubin, CRP, ferritin and sigma of IQC2 for ALP, ALT, AST,CK,CK-MB, ıron, phosphorus,GGT, glucose, calcium, total cholesterol, HDL,LDL, LDH, magnesium, total protein, triglyceride, uric acid, amylase, lipase, total bilirubin,CRP, ferritin tests were≥6.
CONCLUSIONS:Six Sigma Methodology is a very effective method for assessing the laboratory's analytical process performance.In our study, the performance of our laboratory was found to be good or worldclass.
Keywords: six sigma, internal qality control, Allowable Total Error

O-081
Evaluation of analytical quality of cardiac biomarkers in the emergency laboratory by sigma metrics

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OBJECTIVES:The Six-Sigma Methodology is a quality measurement method in order to evaluate the performance of the laboratory. In the present study, we aimed to evaluate the analytical performance of our emergency laboratory by using the internal quality control data of cardiac biomarkers and by calculating process sigma values
MATERIALS and METHODS:Biological variation database (BVD) are used for Total Allowable Error (TEa). Sigma values were determined from coefficient of variation (CV) and bias resulting from Internal Quality Control (IQC) results for 4 subsequent months. If the sigma values are≥6, between 3 and 6, and <3, they are classified as »world-class«, »good« or »un -acceptable«, respectively.
RESULTS:Whenthesigmavalueswereanalyzedbycalculatingthemeanof4months, Troponin I (cTnI), CKMB mass, Myoglobin (Mb) were found <3.
CONCLUSIONS:The "poor quality" levels of cTnT, CKMB mass, Myoglobin sigma values, decision is taken for the improvement of cardiac markers in our laboratory. It is possible to determine the test with high error probability by evaluating the fine sigma levels and the tests that must be quarded by a stringent quality control regime. In clinical chemistry laboratories, an appropriate quality control scheduling should be done for each test by using Six-Sigma Methodology.
Keywords: Six Sigma, total allowable error, bias, Cardiac Biomarkers

O-083
Automated Vitamin D immunoassay comparison with LC-MS/MS method

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OBJECTIVES:Serum 25-hydroxy (25-OH) vitamin D is the major form of vitamin D and the best indicator of vitamin D status in human beings. In this study, we compared analytical performance of automated immunoassay method, Roche Elecsys Vitamin D total assay, with the liquid chromatography tandem mass spectrometry (LC-MS/MS).
MATERIALS and METHODS:A total of 80 samples were used to assess vitamin D analytical perfomance. Vitamin D levels were determined in Roche Cobas E602. Results were classified into three groups; vitamin D concentration of less than 20 ng/mL (LOW, n=20), 20-50 ng/mL (NORMAL, n=41), and >50 ng/mL (HIGH, n=19). Serums were stored at -80 °C for 2 weeks until LC-MS/MS analysis. Regression analysis and Bland–Altman plots were used for comparison between methods.
RESULTS:The correlation for all samples was acceptable (n=80, r=0,961). The r value was higher in samples with low vitamin D levels (n=20, r=0.948) as compared to those with normal vitamin D values (n=41, r=0.902) and high vitamin D values (n=19, r=0.715). The mean percent difference of Elecsys was −2.6% compared to LC–MS/MS. The results were linear with slope of 1.055, intercept of 0.833 ng/mL, a correlation coefficient of 0.961, and a mean bias of -2.6% (P<0,0001)
CONCLUSIONS:Our data show that the Roche Elecsys Vitamin D Total Assay has good correlation with LC-MS/MS. Although the LC-MS/MS method is considered reference method, it needs a special instrument and personnel and is thus expensive. Therefore, Roche's automated immunoassays for vitamin D total assay is more suitable for evaluating vitamin D status.
Keywords: 25-hydroxyvitamin D, correlation, analytical perfomance, electrochemiluminescence, liquid chromatography tandem mass spectrometry

O-084
Calculation of measurement uncertainty of three different biochemistry parameters

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OBJECTIVES:Measurement uncertainty is a quality indicator which is used to show the distribution level of the test result. In principle, two approaches can be used to calculate measurement uncertainty: "bottom-top" and "top-down".The "top-down" approach uses laboratory test performance information, such as intra-laboratory and inter-laboratory quality control data to estimate uncertainty associated with the test results. The aim of this study; to calculate the measurement of uncertainty values of three biochemistry parameters separately using internal and external quality control data. then these values will be compared with the CLIA's% total permissible error (% TEa) values.
MATERIALS and METHODS:In this study, the uncertainty estimation of serum ALP GGT CK levels based on the "top-down" approach described in the Nordest guideline was used as a practical example. The tests were performed by enzymatic method on Beckman Coulter AU5800 analyzer.
RESULTS:SerumALPGGTandCKanalysismeasurementuncertaintywasfoundto be ALP:18.74%, GGT:13.3%, CK:17.7% in the 95% confidence interval,and these values did not exceed the CLIA% TEa (ALP:% 20,GGT:%15,CK:%20) values. The %CV values of the tests were ALP control level 1% CV: 7,07 level 2% CV: 5,36, GGT level 1 % CV: 2,36 level 2 %CV: 3,45, CK level 1 %CV: 5.58 level 2% CV: 2.9 and bias values were determined as bias(ALP): 0.6, bias(GGT): 2.8, bias(CK): 4.79.
CONCLUSIONS:The uncertainty value is a parameter that increases confidence in the accuracy of the measurement results.Therefore, laboratories should

provide results that do not exceed the target measurement uncertainty values.
Keywords: Measurement uncertainty, Accuracy

O-085
Development of a LC/MSMS method for quantification of adrenal-derived 11-oxygenated 19-carbon steroids

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OBJECTIVES:Recent studies have shown that adrenal-derived 11-oxygenated 19-carbon (11oxC19) steroids may be associated with congenital adrenal hyperplasia (CAH) as well as premature adrenarche, polycystic ovary syndrome and castration resistant prostate cancer. In our study, we measured 11β-hydroxyandrostenedione (11OHA4) and 11β-hydroxytestosterone (11OHT) metabolites, which are most likely of adrenal origin, by a LC/MSMS method. In addition, we thoroughly validated our method and evaluated whole steroid profile in patients who have 21-hydroxylase deficiency (21OHD) which accounts for the majority of CAH cases.
MATERIALS and METHODS:11OHA4 and 11OHT standards (Steraloids, USA) and 11OHA4-d7 internal standard (Cambridge Isotopes, USA) were used in the preparation of the calibrators and internal standard working solution, respectively. Plasma samples were prepared by liquid–liquid extraction (LLE). Poroshell 120 EC-C18 column (50×2.1mm, 2.7µm; Agilent Technologies, USA) was used and the analysis time was set as 15 min. Precursor and product ions for 11OHA4 (303.2>121.0, 267.0), 11OHT (305.3>121.0, 269.0) and 11OHA4-d7 (310.43>128.0, 243.0) were determined.
RESULTS:The linear measuring range of method was determined as 0.1–20.0 ng/mL for 11OHA4 and 50–1000 pg/mL for 11OHT. The %CV values of the upper and lower limits of the measuring interval were <15%. Two 11oxC19 steroids were significantly higher in 21OHD patients (n=7) compared to controls (n=56) (3–5-fold, $P < 0.0001$).
CONCLUSIONS:Our findings suggest that 11oxC19 steroids might serve as an additional biomarker in patients with 21OHD. LC/MSMS methods which have unique advantages like permitting more flexibility in application of new biomarkers are considered as reference methods for measuring steroid hormones.
Keywords: 11-Oxygenated 19-Carbon Steroids, congenital adrenal hyperplasia

O-086
Structural bioinformatics approach in bioactive peptide research: Tomato vicilin case study

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OBJECTIVES:Bioactive peptides (BAP) are gaining importance due to their proven health benefits. Food proteins are valuable sources for BAPs with antihypertensive (ACE inhibitory), antidiabetic (DPP-IV inhibitory), antioxidant and antimicrobial activities. There are several bioinformatics tools used in identification of BAPs based on amino acid sequence of the protein and the digestion patterns of different proteases. However, these tools do not take into account the tertiary structure of the protein of interest and therefore can not accurately predict the peptides which will be released under experimental conditions.
MATERIALS and METHODS:Tomato seed proteins were extracted using a modified protocol and were analyzed using electrophoresis. The structure of *Solanum lycopersicum* (tomato) vicilin was modeled based on the experimental structure of *Solanum melongena* vicilin using I-TASSER and RaptorX. The models were visualized and analyzed using PyMol Graphics.
RESULTS:Here, we present a structural biology approach to predict BAP release from tomato seed proteins. Among the extracted proteins, vicilin was selected for further analysis. The structure of vicilin was modeled and was subjected to in silico structure based proteolysis. Our approach takes into account the surface accessibility of specific cleavage sites of enzymes; carboxyl terminus of lysine or arginine for trypsin and large hydrophobic or aromatic side chains for chymotrypsin. The resulting peptides are further evaluated using BIOPEP for their ACE and DPP-IV inhibitory activities.
CONCLUSIONS:BAPs are very useful considering their therapeutic potential. Structure based approaches will shed light on time consuming experimental studies in order to produce targeted peptides.
Acknowledgements: This work is supported by TÜBİTAK(117O319)
Keywords: Bioactive peptides, Protein Structure, Food proteins

O-087
***In silico* prediction of antidepressant-binding sites on human glutathione reductase**

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OBJECTIVES:Antidepressants, which are available worldwide, represent widely used treatments for major depressive disorder. They are grouped into various classes of compounds with slightly different mechanisms of action. Glutathione reductase (GR; EC 1.6.4.2) is a homodimeric enzyme that plays a crucial role in the regeneration of the antioxidant “recharger” glutathione (GSH) from glutathione disulfide (GSSG). While reduced antioxidant capacity is associated with many pathologies, cancer cells are known to use diverse strategies to increase their antioxidant capacity. For this reason, GR inhibition is expected to have divergent consequences in human health and disease.
MATERIALS and METHODS:Here, using protein–ligand docking and interaction profiling as well as ligand (un)binding simulations, we aim at predicting the mode of interaction between human GR and six antidepressants: two selective serotonin reuptake inhibitors (fluoxetine and sertraline); two tricyclic antidepressants (amitriptyline and clomipramine); and two alternative or nontraditional antidepressants (hypericin and pseudohypericin). We evaluate our *in silico* data according to *in vitro* results from enzyme kinetic studies previously conducted and reported by our research group.
RESULTS:All the antidepressants in question appear to be accommodated well in an eccentric cavity located in between the two monomers of GR. Hypericin and pseudohypericin, with their large rigid hydrophobic ring systems, bind the enzyme with the highest predicted affinity.
CONCLUSIONS:Overall, these interactions may subject healthy cells and tissues to oxidative stress. On the positive side, however, they may guide medicinal chemists in the search for new anticancer drugs.
Keywords: antidepressants, human glutathione reductase, computational biology, oxidative stress, anticancer drugs

O-088
Smart approval service for biochemical tests

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OBJECTIVES:Biochemical tests’ results have been approved manually by clinical laboratory experts for decades. This process causes a remarkable loss of time for experts; it is highly likely that some of test results are mistakenly approved/unapproved, since massive amounts of test results are produced in every passing moment. This study aimed to automatically evaluate the test results by handling the problem as binary classification in artificial intelligence (AI).
MATERIALS and METHODS:Utilized AI models were data-driven machine learning (ML) approaches (Naïve Bayesian (NB), Support Vector Machine (SVM), Multi-layer Perceptron (MLP)) and expert-cooperated Fuzzy Systems (FSs). Dataset containing total 28 type of biochemical tests employed in one month-period was obtained from Istanbul Provincial Health Directorate, Maltepe (Number 3) Public Health Laboratory. There were 379340 manually approved and 3568 unapproved tests. Considered properties of tests were test-result, gender-of-patient, age-of-sample, has-interference, distance-to reference-range, delta-check, average-of-median, test-repeated, has-previous-test, and previous-test-result.
RESULTS:5-fold cross-validation was applied for each test type and ML

method. Performance evaluation was based on area under the Receiver Operating Characteristic curve (ROC-AUC). Among the ML approaches, NB dramatically outperforms SVM and MLP. Although FSs have lower data-dependency, FS's classification capability was on par with NB (ROC-AUC: 0.9997-0.9996).
CONCLUSIONS:Approval of test results by an automatic decision-making mechanism is essential need to make rapid, efficient and standardized evaluation. In this study, the feasibility of automatic approval process was empirically investigated. The best classification capability was obtained by NB and FSs.
This research was supported by TUBITAK regarding project "3180740-PROBO Smart Approval System".
Keywords: Biochemical tests, laboratory approval system, artificial intelligence

O-089
Evaluation of saliva Kallikrein-8 levels related with stress

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OBJECTIVES:Kallikreins are a group of serine proteases that are enzymes capable of cleaving peptide bonds in proteins. It is a proteolytic enzyme that mediates the conversion of kininogen (α2-globulin) to brandykinin. Kallikrein is found in blood, lymph, saliva and various external secretions. The aim of this study was to determine whether KLK8 changes in saliva due to stress.
MATERIALS and METHODS:The study included 22 students (15 female / 7 male) from Faculty of Dentstry of Cyprus University of Health and Social Sciences, Term-I. General and dental health of the students were evaluated in the appropriate anamnesis format. Saliva samples were collected between 08.00-09.00 am in the morning, before the exam (12.00) and after the exam (14.00-15.00). It was collected by SARSTEDT brand saliva collection tubes as recommended. Kallikrein levels were measured by KLK 8 Human ELISA kit (pg/mL).
RESULTS:Body mass index and mean age (years) of the students were 20.4 ± 0.93 and 21.6 ± 3.46, respectively. In the present study, salivary kallikrein-8 levels were determined morning, before and after the exam. There were a statistically significant differences between salivary samples of kallikrein-8 values in the morning and before/after exam ($p < 0.001$, $p < 0.001$, respectively). However, there was no statistically significant difference between salivary kallikrein-8 before and after the exam ($p = 0.985$). Electrobiochemical studies are ongoing to confirm the results
CONCLUSIONS:These results show that KLK8 changes in saliva due to stress.
Keywords: Kallikrein-8, Saliva, Stress

O-090
The evaluation of ADAMTS-1 and ADAMTS-13 levels at coronary collateral circulation

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OBJECTIVES:Coronary collateral circulation (CCC) plays important roles at coronary artery disease. It was revealed that ADAMTS-1, ADAMTS-13 and VEGF are included at angiogenesis and arteriogenesis. We aimed to show the relationship between ADAMTS-1, ADAMTS-13, VEGF levels and grade of CCC.
MATERIALS and METHODS:The patients who were applied to underwent coronary angiography according to European Society of Cardiology guide, were included in study. Collateral degree was graded according to Rentrope Cohen classification. Patients who had grade 0-1 collateral vessels were classified poorly-developed collateral group; grade 2-3 collateral vessels were classified well-developed collateral group. VEGF, ADAMTS-1 and ADAMTS-13 levels were measured by ELISA.
RESULTS:From patients who had >%90 obstruction at Coronary Angiographic View,36 patients were at well-developed collateral group and 33 patients were at poorly-developed collateral group. There has been no stastically significant difference between groups ADAMTS-1, ADAMTS-13 and VEGF levels (p=0,428, p=0,577, p=0,450). On the other hand, ADAMTS-1 levels were lower in well-developed collateral group (6.6±6.4) than poorly-developed collateral group (9.6±11.9).
CONCLUSIONS:According to our results, VEGF levels of patients with Coronary Artery Disease (CAD) were higher than the normal population. In addition, plasma VEGF level seems not to be associated with development of CCC. The alteration of ADAMTS-1 might have role the formation of CCC, but ADAMTS-13 level may not be associated with development of CCC. Our data highlighted that ADAMTS-1 and ADAMTS-13 molecules cannot be used as predictor marker for CCC. Further studies with more participants will elucidate the role of ADAMTS-1 and ADAMTS-13 on the development of CCC.
Keywords: ADAMTS-1. ADAMTS-13. Coronary Collateral Circulation

O-091
Apelin and other adipokines as potential biomarkers in myocardial ischemia

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OBJECTIVES:Apelin is an endogenous peptide and ligand of the G protein-coupled receptor (also known as the APJ receptor). Apelin plays role in cardiovascular systems and participates in pathological processes for heart failure, obesity, diabetes. Apelin is called new adipokine and can be secreted by fat cells. Adipose tissue express and secrete numerous adipokines. CTRP1 and CTRP9 novel members of the adipokine family, have intersecting functions in the regulation of lipid metabolism and contribute to cardiovascular protection. In this study, we investigated the association of serum levels of apelin, CTRP1 and CTRP9 with patients with and without myocardial ischemia after myocardial perfusion scintigraphy.
MATERIALS and METHODS:This study was carried out in 44 patients with myocardial ischemia and 44 patients without myocardial ischemia after myocardial perfusion scintigraphy. Serum apelin, CTRP1 and CTRP9 levels measured with ELISA method, whole blood HbA1c measured with HPLC method.
RESULTS:According to t test results for groups; there was a statistically significant difference between the groups in serum apelin, CTRP1, CTRP 9 levels and whole blood HbA1c levels. Serum apelin, CTRP1, CTRP9 levels and whole blood HbA1c levels were found statistically significant between groups ($p = 0.050$, $p = 0.045$, $p = 0.043$ and $p = 0.001$, respectively).
CONCLUSIONS:Our data showed serum apelin, CTRP1 and CTRP9 could be used as potential biomarkers or supportive parameters for myocardial ischemia, and HbA1c, consequently diabetes, may be predisposing factor for myocardial ischemia.
Keywords: apelin, ctrp1, ctrp9, hba1c

O-092

Relationship between platelet activating factor acetylhydrolase and cardiac valvular calcification in dialysis patients

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OBJECTIVES:The cardiovascular mortality risk has increased seriously in Chronic Kidney Disease (CKD), especially dialysis patients. PAF-acetylhydrolase is an enzyme that hydrolyzes platelet activating factor (PAF). Atherosclerosis was associated with valvular calcifications and PAF-AH. So we investigated the association of PAF-AH activities with valvular calcification in dialysis patients.
MATERIALS and METHODS:92 patients treated with hemodialysis (HD) and peritoneal dialysis (PD) and 86 CKD patients which were divided into five groups according to GFR [grade 1-2(> 60), grade 3a(45-59), grade 3b(30-44), grade 4(15-29) and grade 5(<15)] were included in the study. Echocardiography was performed to assess valvular calcification. We analysed urea, creatinine, uric acid, protein, albumin, ALT, ALP, calcium, phosphate, magnesium, cholesterol, triglyceride, HDL, 25-OH D vitamine, iPTH, CRP, NT-proBNP in serum and protein, albumin, creatinine in urine. PAF-AH activity was determined by color change based on the reaction of DTNB with free thiols which formed due to of 2-thioPAF by PAF-AH and measured at 412 nm by a rate method.
RESULTS:There was no significant difference between the PAF-AH activities of dialysis and control groups ($p > 0.05$). Higher PAF-AH activities in HD patients were associated with both valvular calcification and aortic valvular calcification ($p < 0.05$). There was no association between PAF-AH and calcification in PD and control groups.
CONCLUSIONS:In addition to anti-inflammatory and antioxidative properties of PAF-AH, proatherogenic and proinflammatory products formed by enzyme complicates the interpretation of activity changes. Findings in our study suggest that the elevation of PAF-AH activities in HD patients are associated with valvular calcification particularly in aortic valve involvement.
Keywords: Hemodialysis, peritoneal dialysis, PAF-AH, calcification

O-093

Determination of ADMA and ghrelin levels as a marker of endothelial dysfunction in asthma patients

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OBJECTIVES:Asthma is a chronic inflammatory disorder defined as obstruction and hyperresponsiveness of airways. Appetite modulating hormone ghrelin plays a role in various diseases associated to inflammation. Ghrelin regulates secretion of proinflammatory cytokines and induces anti-inflammatory profile, although the underlying mechanisms remain elusive. Nitric oxide (NO) plays important roles such as airway smooth muscle relaxation, airway mucus secretion, and host defense in the lung. Asymmetric dimethylarginine (ADMA) that is defined as a marker of endothelial dysfunction is a competitive inhibitor of NO synthesis. To date, the role of ADMA in the pathogenesis of asthma has not been elucidated completely. The aim of the study was to evaluate ADMA and ghrelin levels in asthmatic patients compared to healthy subjects.
MATERIALS and METHODS:Thirty-eight asthma patients and twenty five healthy controls were included in the study. The patient group was constituted according to the Global Initiative for asthma guidelines. Serum ADMA levels were determined by HPLC and ghrelin levels were measured by ELISA.
RESULTS:Serum ADMA levels were significantly higher in the patients compared to controls ($p=0.014$, $p<0.05$). The median ADMA levels were found about 0.56 µmol/L in patient group. In contrast, ghrelin levels (116.24±2.03pg/ml) were lower in the patients compared to the controls (154.3±21.6pg/ml, $p<0.05$).
CONCLUSIONS:Our findings demonstrated that increased ADMA and decreased ghrelin levels may be contributed to asthma pathophysiology. Our data support the idea that asthmatic patients have risk of endothelial dysfunction for cardiovascular diseases. However, more studies are required to elucidate the molecular mechanisms of ADMA as well as ghrelin actions in asthma.
Keywords: Asthma, asymmetric dimethylarginine, endothelial dysfunction, ghrelin

O-094

Antimicrobial and antioxidant activities of Lactarius deliciosus

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OBJECTIVES:Fungi, which are of particular importance in the ecosystem because of their biodegradable properties, are known to be an important source of biologically active components of both food and medical value. Fungal extracts are used in the treatment and prevention of many diseases. Lactarius deliciosus mushroom is also known as Kanlica mushroom and is an edible mushroom with high nutritional value. The aim of this study was to determine the antioxidant and antimicrobial activities of water, ethanol and cloform extracts of Lactarius deliciosus.
MATERIALS and METHODS:Antioxidant activities of the extracts were determined by DPPH (2,2-diphenyl-1-picrylhydrazase) method. Antimicrobial activities against Escherichia coli, Candida albicans, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa were determined by disc diffusion method.
RESULTS:The water and ethanol extracts of Lactarius deliciosus were found to have antioxidant activity. And also antimicrobial activity was determined according to the solvent used and the type of microorganism. The most susceptible strain was P. aeruginosa and the most resistant strain was E.coli.
CONCLUSIONS:Mushrooms are highly important due to their properties such as fat, vitamins, carbohydrates and proteins. It is important to evaluate these fungi in terms of antioxidant and antimicrobial activity.
Keywords: Lactarius deliciosus, Antimicrobial Activity, Antioxidant Activity

O-095

Telmisartan and irbesartan alleviate methylglyoxal-induced elevation of MG-H1 in VSMCs

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OBJECTIVES:Methylglyoxal (MGO) is a glycolysis by-product and was found elevated in diabetics. It was described as the most powerful glycating agent which results in enhanced AGEs formation. MGO-derived the most important physiological AGE is hydroimidazolone 1 (MG-H1). Telmisartan and irbesartan were suggested to be protective against MGO. The aim of this study was to evaluate the effects of telmisartan and irbesartan on MGO-induced MG-H1 levels under low and high glucose media in vascular smooth muscle cells (VSMCs), those play a prominent role in vascular diseases.
MATERIALS and METHODS:Primary cultured VSMCs were isolated from rat aorta. MGO-treated cells (200 µM) were incubated in low and high glucose media for 48 hours with or without telmisartan or irbesartan (both 10 µM). MG-H1 was measured by ELISA technique as triplicates.
RESULTS:MGO raised MG-H1 concentration in low and high glucose media. High glucose alone elevated MG-H1 levels similar to MGO treatment in low glucose media. While telmisartan and irbesartan did not mitigate MGO-induced MG-H1 increase in low glucose media, they both displayed a significant reduction in MG-H1 concentration in high glucose media. Though telmisartan seemed better to reduce MG-H1 level, there was not any significant difference between telmisartan and irbesartan.
CONCLUSIONS:Our findings showed that telmisartan and irbesartan were effective against MGO-induced MG-H1 concentration increase in high glucose media. The reason could arise from MG-H1 level was highest in MGO-induced

cells cultured in high glucose. Further studies supporting our data are needed and these may guide clinicians to choose the best antihypertensive medicine in diabetic patients.
Keywords: methylglyoxal, MG-H1, telmisartan, irbesartan, high glucose.

O-096
Reelin enzyme levels in suicide or self harm attempt emergency service patients

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OBJECTIVES:Suicide is an important public health problem in our country as in the whole world. Although studies have shown that Reelin enzyme plays a role in the pathophysiology of neuropsychiatric diseases, information on how these enzyme levels change in suicidal behaviour remains unclear. For this reason, we aimed to investigate the enzyme levels of Reelin in self-harm or suicidal patients.
MATERIALS and METHODS:This work includes 86 consecutive suicide patients who applied to Fırat University, Faculty of Medicine, Emergency Medicine Department and 100 healthy age and sex-matched control. Reelin levels were analysed in serum samples in accordance with the ELISA kit procedure. In addition, the effect of demographic data such as body mass index (BMI) and age on Reelin levels were also investigated.
RESULTS:While in the suicide patient group Reelin enzyme level was 3038.31 ng/L, it was 2271.20 ng/L in the healthy control group. When the demographic data were compared with Reelin enzyme levels, it was found that Reelin enzyme levels were negatively correlated with both BMI ($r=-0.298$, $p<0.001$) and age ($r=-0.362$, $p<0.001$).
CONCLUSIONS:It was suggested that increases in serum Reelin enzyme levels may be helpful in the diagnosis of suspicious suicidal cases. Interestingly, people with lower BMI and younger age have higher Reelin levels which calls for further research. When elaborating results on suicidal patients, it should be remembered that too many environmental and/or social factors are effective in suicidal behavior.
Keywords: Suicide, Reelin, Emergency Service, ELISA

O-097
Relationship between lipoprotein(a) and other lipids in children

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OBJECTIVES:Lipoprotein(a) (Lp(a)) levels are hereditary and essentially depend on the Apo(a) gene located on chromosome 6. Lp(a) may cause inflammation, oxidative stress, fibrinolysis and plaque instability. The aim of this study was to determine the correlation of Lp(a) with total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and trİglycerides (TG) in children.
MATERIALS and METHODS:The study included 25 patients with Lp(a) >100 mg/dL, 25 patients with 50-100 mg/dL, 25 patients with 30-50 mg/dL and 25 healthy children for the control group. Fasting blood Lp(a) was measured by immunoturbidimetric method and TC, TG, HDL-C was measured by photometric method in Beckman Coulter AU5800. LDL-C was calculated by the Friedewald formula.
RESULTS:In this study, Lp(a), TC, LDL-C, TG were significantly higher in all patient groups compared to the control group ($p<0.001$). There was no significant difference in HDL-C ($p>0.05$). Only Lp(a) showed statistical difference between the groups ($p<0.001$). No significant difference was found in lipid profile ($p>0.05$). Lp(a) showed a weak positive correlation with TC, LDL-C and TG ($r=0.340$, $p<0.001$; $r=0.326$, $p<0.001$; $r=0.275$, $p<0.001$).
CONCLUSIONS:Lp(a) is an independent risk factor for premature cardiovascular disease. But also it shows a correlation with other cardiovascular risk factors such as TC, LDL-C and TG.
Keywords: Lipoprotein(a), LDL-cholesterol, triglycerides

O-098
Relationship between B-HCG and LUC% levels

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OBJECTIVES:In this study, we aimed to investigate the changes in hematological parameters in pregnancy.
MATERIALS and METHODS:Hematology parameters of pregnant (521 patients) (17180 ± 3652 mIU/mL) and non-pregnant (498 patients) (6.23 ± 0.91 mIU/mL) women were compared. BHCG levels were determined with Siemens Centaur XP and hematological parameters were analysed with ADVIA 2120i Hematology System.
RESULTS:LUC%(pregnant:1.57±0.59,non-pregnant:1.77±0.80;p=0.001),delta neutrophil index (DNI) (pregnant: 2.07 ± 0.51, non-pregnant: 2.49 ± 0.43; p = 0.001); WBC (pregnant: 8.25 ± 1.52 x103/uL, non-pregnant: 7.31 ± 1.64 x103/uL; p = 0.001); neutrophil % (pregnant: 66.54 ± 8.67, non-pregnant: 62.0 ± 9.31; p = 0.001); monocyte % (pregnant: 4.87 ±, 1.32, non-pregnant: 5.27 ± 1.5; p = 0.001); eosinophil % (pregnant: 1.67 ± 0.39, non-pregnant: 2.05 ± 0.73; p = 0.001); basophil % (pregnant: 0.31 ± 0.18, non-pregnant: 0.41 ± 0.04; p = 0.001); lymphocyte % (26.02 ± 5.66, non-pregnant: 28.49 ± 4.68; p = 0.001) showed significant difference.
CONCLUSIONS:Large unstained cells are reported in routine complete blood cell (CBC) tests. LUC are large peroxidase-negative cells, which do not fit into other subtypes of leukocytes and they usually include virally activated lymphocytes, plasma cells, hairy cells, pediatric lymphocytes and peroxidase-negative lymphoblasts. In our study, LUC and DNI values were lower in pregnant women than non-pregnant women. Immune alterations with pregnancy may impair pathogen clearance, resulting in increased severity of disease for several pathogens. Therefore, we hypothesized that LUC value would be increased in infection and it can be a potential useful marker to differentiate infection. It can be suggested that there is resistance to inflammation during pregnancy.
Keywords: inflammatuary marker; LUC%; pregnanant

O-099
Biological variation of beta-trace protein, a novel marker for eGFR along with traditional markers

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OBJECTIVES:Objective evaluation of a test result by the clinician, the establishment of an analytical performance goal or deciding the appropriateness of population-based reference range require the knowledge on the biological variation of the test. In the study, it is aimed to determine the biological variation of beta-trace protein (BTP), a relatively filtration marker for glomerular filtration rate (GFR) along with the conventional markers such as creatinine and cystatin c.
MATERIALS and METHODS:Twenty-two participants aged between 25 and 57 were included in the study. “European Federation of Laboratory Medicine Biological Variation-WG” recommendations were followed. Whereas creatinine levels were analyzed using an autoanalyzer (AU5800,Beckman Coulter Inc.,USA), cystatin C and BTP were measured by nephelometric method

(Atellica NEPH630 System,Siemens Healthineers,Germany). Intra (CVI) and inter-individual (CVG) biological variations for each parameter were calculated, reference change values (RCV), and individuality indexes were determined from these data.
RESULTS:CVA, CVI, and CVG values were as follows, respectively: 5.56/3.31/14.50 for creatinine; 3.48/3.15/12.24 for cystatin C, 5.37/9.91/14.36 for BTP. RCV values for creatinine, cystatin C, and BTP were calculated as 17.94/13.01/31.24, while individuality indexes were found to be 0.23/0.26/0.69, respectively.
CONCLUSIONS:To our knowledge, the study is the first study in literature in which the biological variation of BTP is determined. The closeness of CVI and CVG results for BTP might be interpreted as the molecule not having a substantial natural variation or significant individual characteristics. Because of the high individuality of creatinine and cystatin C tests, using RCV values instead of population-based reference ranges would be more useful in monitoring patients.
Keywords: Biological variation, Beta trace protein, Creatinine, Cystatin C, reference change value

O-100
Calculation of APTTandPT reference intervals from patient data and evaluation of preoperative test utilisation in surgical patients

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OBJECTIVES:The purpose of this study was to verify the reference intervals of our own laboratory by indirect procedure for activated partial thromboplastin time (APTT) and prothrombin time (PT) and investigate whether preoperative coagulation test requests are necessary.
MATERIALS and METHODS:Bhattacharya procedure was used for determination of reference intervals from data of outpatient clinics between January 2017 and June 2019. We eliminated the test requests made by clinics of Child and Adult Emergency Department, Anesthesia and Reanimation, Obstretrics and Gynecology, Nephrology, Infectious Disase, Child and Adult Hemotology outpatients, inpatients and intensive care units. To determine the appropriate test utilisation, preoperative APTTandPT requests were used between July 2018 - June 2019 (n = 4751). Cardiology, Cardiovascular surgery and Oncosurgery patients and repeated test requests were excluded. We evaluated preoperative test requests of APTT and PT by using reference intervals that we created from our own hospital data.
RESULTS:Reference intervals of APTT (sec) and PT (sec) for 1-3, 4-6, 7-9, 10-12, 13-18 age groups were 23.98- 34.87; 11.32-14.45, 24.10-33.08; 11.69-14.37, 24.45-34.67; 11.73-14.38, 24.87-33.90; 11.82-14.45, 24.23-35.18; 11.87-14.42, respectively. Adult reference intervals were for 18-39, 40-49, 50-59, 60-69, 70-79, 80 and above age groups were 24.06-33.63, 11.56-14.87; 23.28-32.40, 11.12-14.07; 23.88-11.09-14.38; 23.62-32.55,11.17-14.65; 23.10-32.43,11.3-14.59; 22.73-35.29, 11.35-14.81 respectively. In pediatric patients 77.1% of APTT and 61.4% PT results; in adult patients 75.8% of APTT and 60.9% of PT results were within the reference ranges.
CONCLUSIONS:A large proportion of preoperative coagulation tests are found within reference ranges and these test requests are mostly unnecessary, time consuming and high costly activities.
Keywords: preoperative tests, hemostasis, reference ranges

O-101
ICD code specific normal ranges are needed, particularly in total bilirubin in this case

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OBJECTIVES:Two healthy young patients admitted to the hospital to obtain a formal report of health. Their total and direct bilirubin levels were slightly higher than the upper reference level so a call was made to the laboratory to investigate the suspicious results.
MATERIALS and METHODS:Internal quality results are validated daily for each instrument in our laboratory and they are nearly perfect; while external quality results for the previous month were also satisfactory. The total and direct bilirubin analysis were repeated in another auto-analyzer but there was no change in the results..
RESULTS:These two individuals were recorded in the laboratory information system (LIS) with a specific ICD code Z02 namely “Encounter for administrative examination”. Prior to medical health check, all nominees had to pass a written exam followed by a physical exam including a long distance running. A pubmed search with key words “sports” and “hemolysis” defined a rise in bilirubin levels of athletes.
CONCLUSIONS:The elevation in bilirubin is thought to be caused by mechanical factors, and named as “marching hemolysis”. The literature suggests to use a dedicated reference range for total bilirubin concentration in relation to the group of athletes. This case is an evidence based application to use reference intervals dedicated to individuals defined by the mentioned specific ICD code. Unfortunately, we can only define reference intervals for age and gender with the LIS we currently use just like the ones we used before which is a limitation.
Keywords: ICD codes, reference ranges

O-102
Pending laboratory tests at discharge in emergency department

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OBJECTIVES:Emergency department (ED) is a department that requires rapid, accurate and effective intervention to patients. However, it is frequently encountered that patients are discharged before all tests are resulted. Laboratory test results pending at discharge (TPAD) from emergency department is a major patient safety concern and can have major adverse health outcomes. The goal of this study is to evaluate the TPAD ratios and factors that affect this situation in our hospital.
MATERIALS and METHODS:Two groups are established as TPAD and tests reported before discharge(TRBD) from the patients admitting to ED and having laboratory test requisition.
RESULTS:Total number of patients with test requisition were 13347, and total number of tests were 34104.
TPAD ratio is detected as 16,4%.TPAD is more frequent in biochemical tests(19,5%), hormones(42,4%), urine tests(18,7%), cardiac markers(21,4%) and coagulation tests(27,1%), while it is less frequently encountered in hematology tests(10%), blood gas analysis(4,6%), and blood ethanol levels(10,9%).
Consideration of delaying tests, regarding the determined test completion time for requestedfromED,showed5,5%delayinTPADwhileitwas1,7%inTRBD($p<0,001$).
Regarding the referral status and reporting the tests before discharge of 370 results with elevated Troponin tests, TPAD ratio was 12,1%(n=38) in non-referred patients and 3,5%(n=2) in referred ones.
CONCLUSIONS:Current study indicates that patient discharge ratios before evaluating the test results is high. This situation endangers the safety of both the physician and the patient. Discharging the patients before the laboratory test are reported, especially the troponin levels in myocardial infarction suspicion, may give rise to irreversible results.
Keywords: Pending tests, laboratory

O-103
The effect of blood lactate levels on mortality in patients with sepsis

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OBJECTIVES:Severe sepsis and septic shock are one of the major causes of mortality in intensive care units, and elevated blood lactate levels are an important indicator of mortality. In this study, we aimed to investigate the effect

of blood lactate values on mortality rate of patients in intensive care unit.
MATERIALS and METHODS:The files of 74 patients diagnosed with sepsis since 01/01/2018 in the Reanimation Intensive Care Unit of Konya Training and Research Hospital were investigated retrospectively.
RESULTS:The mean age was 67.1±15.2 years in the group with mortality within 30 days (n: 45) and 64.9±20.1 years in the group without mortality (n: 29). There was no difference between the groups in terms of age and gender (respectively, p=0.76, p=0.88). The mean duration of hospitalization in patients with sepsis was 12.3 ± 11.1 days and 60.8% of them died. In the group with mortality, the input lactate value was 4.2±3.9 mmol/L and the output lactate value was 7.3±5.4 mmol/L. In patients who were discharged, the input lactate value was 2.5±1.7 mmol/L and the output lactate value was 2.3±3 mmol/L. In our study, we found that the last measured lactate levels of the patients in the intensive care unit were higher than the first measured lactate levels in the group with mortality and this difference was statistically significant (p=0,000).
CONCLUSIONS:Lactate was an independent predictor of sepsis prognosis. Serial lactate monitoring helps to identify patients at high risk of developing mortality and is important for assessing the adequacy of treatment given to these patients.
Keywords: Sepsis, mortality, lactate

O-104
The anti-inflammatory effects of orexin receptor antagonist on endotoxemia induced sepsis model

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OBJECTIVES:Inflammatory diseases, including sepsis, are often accompanied by loss of appetite. As an orexigenic peptite, orexin increases appetite; however little is known about its role in sepsis related inflammatory conditions. Thus, the aim of the study is to investigate the role of orexin in Escherichia coli lipopolysaccharide (LPS) induced endotoxemia by using its dual receptor antagonist almorexant.
MATERIALS and METHODS:Sprague Dawley rats (male=female; 250-300g) were used: (1) Control and (2) Endotoxemia (E) groups were treated with saline; (3) E + orexin antagonist group was treated with almorexant (30 mg/kg ip) for 3 days. On the 4th day, saline (control group) or LPS (others) was injected. Six hours after LPS injection, rats were sacrificed; their trunk blood, duodenum, stomach, liver, colon and kidney samples were collected. Tissue samples were analyzed for myeloperoxidase (MPO) activity, malondialdehyde (MDA) and glutathione (GSH) levels and microscopic damage was scored. Cortisol, tumor necrosis factor (TNF)-α, Interleukin (IL)-1β and IL-6 levels were measured in serum samples.
RESULTS:Endotoxemia increased MPO activity, MDA levels in all tissues and caused GSH depletion. MPO activity and MDA levels in all tissues and, cortisol, TNF-α, IL-1β and IL-6 levels in serum were decreased with almorexant injection compared with the endotoxemia group. Microscopic damage scores also reduced. However, almorexant treatment could not prevent GSH depletion induced by endotoxemia.
CONCLUSIONS:The results of our research showed that almorexant has anti-inflammatory effects on LPS induced sepsis. Probably, dual orexin receptor antagonist, almorexant showed its anti-inflammatory effects by inhibiting tissue neutrophil infiltration and preventing lipid peroxidation.
Keywords: Orexin, Almorexant, Sepsis, Endotoxemia, inflammation

O-105
Correlation of CRP with blood-based inflammatory markers; large cohort study

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OBJECTIVES:The aim of the study is to examine the relationship between CRP and several blood-based markers (neutrophil/lymphocyte, platelet/lymphocyte, lymphocyte/monocyte, monocyte/HDL ratio), which have recently become popular in a large population of patients.
MATERIALS and METHODS:Samples were taken at Amasya Central Public Health Laboratory between 01.01.2018-31.12.2018 and the results of n=26,691 (18,243 females, 8,448 males) patients were screened retrospectively. The correlation between CRP and neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), lymphocyte/monocyte ratio (LMR), monocyte/HDL ratio (MHR) were determined using the SPSS 15 for Windows program.
RESULTS:According to the results of the study, there was a positive correlation (r: 0.301, p <0.01, r: 0.180, p<0.01, r: 0.305, p<0.01, respectively) between CRP and NLR, PLR, and MHR, while there was a negative correlation (respectively: r: -0.224, p<0.01, r: -0.102 p<0.01) between CRP and LMR and HDL.
CONCLUSIONS:Based on the positive correlation (excluding LMR) of the rates of hematologic parameters and CRP, a marker of classical inflammation, we think that these rates can be used as a supportive marker for inflammation in patients under CRP follow-up. However, we believe that these rates should be supported by further studies to be conducted in various patient groups.
Keywords: inflammatory markers

O-106
Serum cytokine and complement levels in amyotrophic lateral sclerosis and their association with LRP antibody positivity

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OBJECTIVES:It has been known that neuroinflammation plays an important role in the pathophysiology of amyotrophic lateral sclerosis (ALS), and few anti-neuronal antibodies have been detected in some studies. The aim of this study was to investigate the role of primary T helper subset Th1 (IFN-gama), Th2 (IL-4) and Th17 (IL-17) cytokines and their association with complement factors.
MATERIALS and METHODS:25 patients with ALS (mean age 56.5±6.9 years;16 males/9 females) and 25 healthy controls (58.7±8.5 years;14 males/11 females) were included in the study. Serum C1q, C3, IL-4, IL-17 and IFN-gama levels were measured by ELISA and LRP4-antibody was demonstrated by immunofluorescence method performed with HEK cells transfected with the plasmid encoding LRP4.
RESULTS:Serum C1q and C3 levels were lower and IL-17 levels were higher in ALS patients compared to healthy controls. There was no significant difference between IL-4 and IFN-gama levels. In spinal-onset ALS patients serum C1q and C3 levels were higher than those with bulbar onset. LRP4 antibody was found in 4 cases. All patients with LRP4 antibody positive sera had spinal onset. C1q and C3 levels were significantly higher in the LRP4 antibody positive sera. In primer neuron culture studies, only LRP4 antibody-positive ALS serum IgG molecules binded neurons.
CONCLUSIONS:Our results support that the predominant role of Th17-type immunity in ALS. The increase of complement factors in spinal-onset cases suggests that the complement system is involved in pathogenesis of these patients. Conceivably, LRP4 antibodies might bind LRP4-expressing motor neurons thereby activating the complement system and thus contributing to motor neuron destruction.
Keywords: Amyotrophic Lateral Sclerosis, complement factors, Cytokine, LRP4 antibody

O-107
The relationship between standard sedo-analgesia implementation and serum procalcitonin levels in intensive care unit

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OBJECTIVES:Inflammatory pain is a common symptom in intensive care unit (ICU) patients. Procalcitonin levels increase in systemic inflammation. The aim of this study was to determine the relationship between serum procalcitonin levels and standard sedo-analgesia in the control of inflammation-related pain levels in the ICU patients.
MATERIALS and METHODS:The study included 69 patients hospitalized to the Anaesthesiology and Reanimation ICU of Manisa Celal Bayar University. Patients are divided into two groups; Group 1(n = 36): within two months before, and Group 2(n = 33): within two months after standard sedo-analgesia protocol was implemented. Before the implementation of the sedo-analgesia protocol, patients were treated based on the subjective evaluation of physicians and nurses at irregular intervals. After the implementation of sedo-analgesia protocol, pain and sedation requirements, were evaluated regularly with reliable scales, and treated with appropriate prescribed drugs and doses. Serum procalcitonin levels were daily measured for five days. Serum procalcitonin levels were daily measured for five days with Cobas e411 autoanalyser.
RESULTS:Procalcitonin difference between day 1 and day 5 was analyzed in both groups and no statistically significant difference was found between Group 1 and Group 2 (p=0.41). When the 5-day procalcitonin values of both groups were compared, no strong correlation was observed (r=0.412, -0.150, 0.053, 0.365, 0.291, respectively).
CONCLUSIONS:Procalcitonin did not show a different course in the five-day follow-up with the start of our sedo-analgesia protocol. Thus, we conclude that procalcitonin may not be used as a biomarker to monitor the standart sedo-analgesia protocol.
Keywords: Pain, Procalcitonin, Sedation, Analgesia, Inflammation

O-108
Midkine can not be accepted as a new biomarker for the diagnosis and the treatment of unexplained female infertility

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OBJECTIVES:This study aimed to investigate whether a growth factor and a cytokine midkine (MK) can be a new biomarker for the diagnosis and the treatment of unexplained female infertility (UFI) cases.
MATERIALS and METHODS:Serum (S), follicle fluid (FF) and cumulus cells (CCs) of the patients aged 20-45 years, diagnosed with male factor (MF), UFI and polycystic ovary syndrome (PCOS) and undergoing Intracytoplasmic Sperm Injection (ICSI) procedure were used. The Anti-Müllerian hormone (AMH) and MK levels with other hormone levels, the oocyte and embryo qualities, the fertilization and pregnancy rates, and cumulus cells (Number, ultrastructure, and apoptosis) were evaluated. Student-T-test was performed and $p<0.05$ was considered statistically significant.
RESULTS:The lowest and highest numbers of CCs were found at UFI and PCOS, respectively ($p<0.05$). The lowest viability rate with the highest apoptosis rate was determined at PCOS ($p<0.05$). The lowest apoptosis rate with the highest viability rate was evaluated at MF ($p<0.05$). The ultrastructural evaluation revealed that there were widespread autophagic vacuoles at PCOS and lipid droplets at UFI with MF. CCs with apoptotic appearance was frequently detected at PCOS. Highest AMH and MK levels (S, FF) were found at PCOS; however, the lowest levels of them were detected at UFI ($p<0.05$). These values found at UFI were similar to MF ($p>0.05$).
CONCLUSIONS:MK can not be accepted as a new biomarker for the diagnosis and treatment of UFI.
Keywords: Unexplained female infertility, midkine, anti-müllerian hormone, polycystic ovary syndrome, cumulus.

O-109
Perspective of C-Peptide from diabetes window

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OBJECTIVES:C-peptide indicates endogenous insulin production. It is a good marker for β-cell capacity. In recent years, the use of β-cell capacity for insulin use in the treatment of Type 2 DM has been recommended. According to the C-peptide level, the use of oral antidiabetics has been prescribed. C-peptide is used for monitoring pancreatic capacity. In this study, we aimed to emphasize the importance of C-peptide in the of DM.
MATERIALS and METHODS:The number of C-peptide tests studied by the biochemistry laboratory of our hospital in the last 5 years were classified according to years. Subsequently, the annual changes were grouped as below 1.1, 1.1-4.4 and > 4.4 and % ratios were found.
RESULTS:6794 C-peptide analyzes have been performed in our laboratory in the last 5 years. Percentage distribution of patients by years was found respectively as 11.2, 11.4, 18.0, 23.2, 36.3. The annual percentage distribution of patients with C-peptide <1.1 was respectively 23.2, 42.1, 19.7, 35.0, 10.9. The ratio of patients with C-peptide between 1.1-4.4 were 56.0, 31.4, 62.7, 52.1, 73.6 per years. The ratio of patients with C-peptide> 4.4 to overall patients in the same year was 20.8, 26.5, 17.6, 12.9, 15.4.
CONCLUSIONS:There was a continuous increase in the number of test requests. The C-peptide is used not only for DM classification but also for the follow-up of DM patients. We think that this will reduce the need for parenteral insulin treatment. We think that the clinical laboratory planning should be made accordingly.
Keywords: C-peptide, DM, β-cell

O-110
Development and validation of a biosensor for measurement of serum hypoxia-inducible factor-1

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OBJECTIVES:Normal oxygen delivery is essential for survival. Hypoxia, which is a common feature of various pathological conditions, ranging from cancer to inflammatory diseases, occurs when normal oxygen delivery is altered by an imbalance between cellular oxygen demand and tissue oxygen supply. Among the intricate mechanisms organisms have developed to maintain oxygen homeostasis, a family of hypoxia-inducible transcription factors (HIFs), are found to be the main regulator adaptive cellular response to hypoxia. Although ELISA can be used for its measurement, the lability of the protein and length of the analysis (>5 hours) pose limitations. Thus, our aim is to develop an electrochemical impedance spectroscopy (EIS) based biosensor system for quick and reliable measurement of HIF-1α in tissue.
MATERIALS and METHODS:HIF-1alpha antibodies have been used as a biota receptor. For immobilization, the electrode was first modified with albumin, followed by PAMAM. The new biosensor was compared with the conventional ELISA method.
RESULTS:Based on the chronoimpedance data, total analysis time for EIS was chosen as 15 minutes. Calibration curve was constructed by locating electron transfer resistance on y-axis and HIF1 concentration on x-axis, between 50-1000 pg/mL. LOD and LOQ of the biosensor were calculated as 14.45 pg/mL and 43.8 pg/mL, respectively. The new biosensor showed very good correlation when compared with the conventional ELISA method (R2= 0.99649).
CONCLUSIONS:We developed and analytically validated a biosensor system to measure HIF-1α in serum. This new biosensor promises more timely and accurate measurements in determining the tissue oxygenation in patients who have hypoxia related conditions such as diabetic foot.
Keywords: hypoxia inducible factor 1 alpha, biosensor, impedance, PAMAM

O-111

A fast and convenient UPLC - MSMS method for routine analysis of GALT activity from dried blood spot

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OBJECTIVES:Galactosemia is a disorder of carbohydrate metabolism most commonly caused by galactose-1-phosphate uridylyltransferase(GALT) deficiency.Currently,GALT deficiency screening is performed by fluorometric method from dry blood spots(DBS) and comfirmed by LC/ MSMS from whole blood samples.The aim of our study is to develop a fast,low cost,reliable LC/ MSMS based method for detection of GALT activity from DBS which does not need whole blood samples for verification and to compare the method performance with a commercial fluorometric neonatal GALT kit.
MATERIALS and METHODS:In the developed method,ACQUITY UPLC HSS-T3,2.7 µm,2,1x50mm column was used as the stationary phase and 5mM ammonium formate in acetonitrile/water 50/50% was used as the mobile phase. Injection volume was 5µL while flow rate was 0.4mL/minute.Mass spectrums were determined with Waters Xevo TQD MS/MS system.
RESULTS:The method has been fully validated to ensure good selectivity,a satisfactory detection limit at 6.2nM for UDP-Galactose, acceptable intra- and inter-day accuracy and high precision.A linear response function was established for the range of concentrations between 0.05 –100µM(R^2= 0.9992) for ^{13}C6-UDPGalactose.Controls' enzyme activity levels were clearly distinguishable from patients' levels ($p<10^{-7}$) with a mean value of 42.29±19.73µmol/gHb/h (n=50) for controls and 0.03±0.025 µmol/gHb/h for patients (n=7).Recovery was found as 88% for low QC and 89% for high QC whereas matrix effect was found as 88% for low QC and 102% for high QC.
CONCLUSIONS:This fast, accurate, reliable and sensitive method to analyze GALT levels with LC-MS/MS system in DBS could contribute to facilitate a national newborn screening program in Turkey.
Keywords: GALT, DBS, LCMSMS, newborn screening, validation

O-112

Magnetic bead based electrochemical food and enzyme activity analysis by using SPE dependent immunosensors

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OBJECTIVES:Gathering magnetic beads (MBs) and screen printed electrode technology allows a highly sensitive and innovative methodology for the development of amperometric immunosensors. These researchs purpose are the detection of β-casomorphine-7 peptide in cheese rennet and analyze the substrate effect on HRP-based amperometric immunosensors by using this combined technology.
MATERIALS and METHODS:Both study were based on direct and competitive immunosensor protocols in which antigen-antibody and labeled enzyme immunoassay procedure was followed. HRP substrates ABTS and TMB were used to measure the HRP activity as the last step of the amperometric immunosensor analysis. Signal of the biosensors was in nano amper (nA) range.
RESULTS:For BCM-7 detection, competitive type immunosensor sensitivity was found in ng/ml range and the detection limits were found as 0.5-200 ng/ml. BCM-7 detection in different commercially available cheese rennets was also done. For HRP-based enzymatic sensor development, it is found that TMB is more sensitive than ABTS as the substrate of the HRP.
CONCLUSIONS:BCM-7 detection in cheese rennet with MB-SPE based immunosensor is the first study in the literature. For the other study, HRP activity detection with immunosensor type biosensor by using different substrates gives us the best substrate for HRP activity determinations in amperometric detection. These studies were supported by Erciyes University Scientific Research Projects Unit under the code of FYL-2018-8413 and Tübitak 1509, 9130058, PrintECELISA project.
Keywords: Amperometric immunosensor, BCM-7, HRP, ABTS, TMB

O-113

Transcriptomic meta-analysis in pancreatic ductal adenocarcinoma reveals therapeutic targets and diagnostic biomarkers

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OBJECTIVES:Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer, which has the highest mortality rate of all solid tumors. The absence of an effective screening process and distinctive symptoms, causes a delay in diagnosis. Traditional chemotherapy and curative surgery have limited benefits on patient survival. Enzymes are one of the most important groups of drug targets and are preferred markers for the detection of various diseases. This study aims to identify up-regulated genes encoding enzymes in PDAC to suggest novel therapeutic targets for more effective treatments to be developed and diagnostic biomarkers for PDAC.
MATERIALS and METHODS:NCBI Gene Expression Omnibus (GEO) was searched for datasets using keywords 'pancreatic ductal adenocarcinoma'. The inclusion criteria were i) Gene expression microarray data, ii) human-derived pancreatic ductal adenocarcinoma tissues and normal pancreatic tissue samples. All data processing and integration procedures were performed using ExAtlas. The false discovery rate is less than 0.05, and the change of gene expression is ≥ 10-fold were considered significant. The up-regulated enzyme-coding genes were detected in the differentially expressed gene list.
RESULTS:The random effect integrative meta-analysis of five submissions (GSE46234, GSE19280, GSE43795, GSE41368, and GSE71989) containing 24 tumor-normal tissue pairs revealed 22 up-regulated genes, two of which encoding enzymes. The enzyme-coding genes with 10-fold differential expression compared to the controls were SULF1 (sulfatase, fold change=22.135) and KYNU (kynureninase, fold change=10.716).
CONCLUSIONS:The results of this study suggest that sulfatase and kynureninase may have the potential to become diagnostic biomarkers and therapeutic targets for PDAC, which merits further investigation.
Keywords: Pancreatic Ductal Adenocarcinoma, microarray, meta-analysis, enzyme, gene expression.

O-114

Assessment of Vitamin D levels in Şanlıurfa region

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OBJECTIVES:The aim of this study was to determine serum vitamin D levels in Şanlıurfa region and to investigate the existence of difference between its level saccordingtoage, sex and season.
MATERIALS and METHODS:Scanning the findings of serum 25-hydroxy vitamin D at software system of Biochemistry Laboratory, Education and Research Hospital, Harran University was applied during the period 01.01.2018 and 01.06.2019. Serum 25-hydroxy vitamin D level had determined by LCMS-8045 liquid chromatography mass spectrometer auto-analyzer. SPSS version 21 program was used to evaluate normality test with Kolmogorov-Smimov test. Descriptive statistics were expressed as median (min-max) since the data were nonparemetic. Mann-Whitney U test andKrusskal-Wallis test were used fo rstatistical difference among the studied groups. Significance level was accepted as P <0.05. Intra-laboratory variation coefficients (CV%) of all methods used were<3%..
RESULTS:In our retrospective study, vitamin D deficiency was found in 68.21% of 6182 patients. In comparison the results according to gender, age, and season, a significant difference (p <0.001) was found in serum vitamin D levels and this was in accordance with the literature.
CONCLUSIONS:As a result, In Sanliurfa, the reference range of vitamin D level was determined according to age, sex and seasonal parameters. Considering the influencing factors, we strongly recommend checking serum vitamin 25 (OH) -D during annual controls and to raise public awareness about importance of sunbathing to prevent vitamin D deficiency.
Keywords: Vitamin 25-OH, Age, Sex

O-115

The role of HDL-associated MPO and PON-1 for coronary artery disease in Hashimoto Thyroiditis

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OBJECTIVES:Hashimoto's thyroiditis is an autoimmune disease of the thyroid gland.Free radicals have been reported to be responsible for the complications observed in the pathogenesis of thyroid diseases and in the later stages of the disease.MPO,which is released during inflammation, is an oxidative enzyme present in phagocytes.MPO could be a key element responsible for oxidative damage in the artery wall.PON-1,which is one of the molecules that play a role in oxidant balance, is an enzyme that has the role of inhibiting lipoprotein oxidation by hydrolyzing lipid peroxides in oxidized LDL structure.We investigated the role of HDL-associated MPO and PON-1 in patients with HT in terms of coronary artery disease
MATERIALS and METHODS:Our study group consisted of 54 patients with Hashimato diagnosis and 28 healty individuals as control group.MPO and PON1 levels were determined spectrophotometrically
RESULTS:When the study groups were evaluated,PON1 levels were significantly lower in patients with hashimato thyroiditis than healthy subjects ($p<0.05$,$p=0.032$).When the study groups were evaluated, MPO levels were significantly higher in patients with hashimato thyroiditis than the control group ($p<0.05$,$p=0.001$).A negative correlation was obtained between MPO and PON1($r=-0.685$)
CONCLUSIONS:The decrease in PON1 activity and increase in MPO activity due to hypothyroid effect increases lipid peroxide formation and accelerates oxiLDL formation,which leads to decrease in antioxidant capacity and development of atherosclerosis.Since oxidative stress in thyroid diseases is also responsible for the complications observed in the later stages of the disease,we think that important data were obtained with this study in terms of both diagnosis and treatment
Keywords: Hashimoto Troiditis, MPO,PON-1,HDL

O-116

Drug-induced (quinine) acute hepatitis with high level of serum vitamin B12

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OBJECTIVES:Acute hepatitis; viruses, drugs, alcohol, metabolic diseases, toxins caused by liver cell necrosis and inflammation of the liver. Since liver plays an important role in the metabolism of drugs, drugs are one of the most common causes of toxic liver damage.The diagnosis of drug-induced hepatitis is usually made by ruling out other causes and it should be remembered in the patient's history.
In this case, a patient who presented to the infectious diseases clinic with fatigue was presented.
MATERIALS and METHODS:Drug-induced liver damage (IBCT) is one of the most common causes of hepatotoxicity
RESULTS:In liver pathologies, ALT, AST, ALP, GGT and bilirubin tests, which are described as liver function tests, are expected to increase or decrease according to the severity of the clinic. What is striking in this patient is the change in vitamin B12 measurements. Although it was initially thought to be the interference of quinine in immunassay methods, a B12-quinine interference was not found in the literature review.
CONCLUSIONS:The elevation of vitamin B12 in liver cell damage suggests that it can be used as a marker in the diagnosis and / or treatment of acute hepatitis. Furthermore, as observed in cirrhosis cases, rapid decrease after elevation in AST and ALT enzymes indicates a poor prognosis and a prolonged tendency to increase in B12 levels may indicate a poor prognosis.
Keywords: Acute hepatitis, vitamin B12, quinine

POSTER PRESENTATION ABSTRACTS

P-001
Determination of serum tryptophan and its' metabolites by tandem mass spectrometry

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OBJECTIVES:Tryptophan (Trp) is an essential amino-acid and the precursor of several biologically active compounds such as kynurenine (KYN) and serotonin (5HT). Its metabolism is associated with various physiopathological conditions, such as cardiovascular diseases, cancer, immunomodulation or depression. Our aim of this study is to determine and validate serum tryptophan and its' metabolites by high performance liquid chromatography tandem mass spectrometry (LC-MS/MS).
MATERIALS and METHODS:100 µL of internal standard (L-Kynurenine-d4) was added to 300 µL serum sample and 1000 µL acetonitrile (containing 1% formic acid) was added as a precipitating agent. Then the mixture was vortexed at 12 000 rpm for 10 min. 1000 µL of the supernatant was transfered into clean glass tubes and evaporated under nitrogen. The residues in the tubes were dissolved in 200 µL acetonitrile: water (25:75, v/v) mixture containing 0.1% formic acid and 40 µL was injected to ABSCIEX API 3200 mass spectrometry. Total run time was 5 minutes
RESULTS:Limit of detection-quantation levels were 1.62-3.25, 1.22-2.44, 0.48-1.95, 0.97-1.94, 1.56-3.12 µg/mL for tryptophan, kynurenine, kinurenic acid, 3-hydroxy kynurenine and 3-hydroxy- anthranilic acid, respectively.
CONCLUSIONS:Evaluation of tryptophan pathway with all metabolites may help to elucidate the role of this pathway in disease pathogenesis. For this purpose, the mass spectrometer technique can be considered as a suitable method.
Keywords: Tryptophan, Tandem mass spectrometry, Pathway

P-002
Determination of tryptophan and kynurenine by LC-MS/MS by using amlodipine as ınternal standard

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OBJECTIVES:Tryptophan (Trp) is an essential amino acid that plays an important role in cell metabolism and Kinurenine (Kyn) is its main metabolic pathway. By using ultra-high-performance liquid chromatography coupled to electrospray ionization triple quadrupole mass spectrometry (UHPLC-ESI-MS/MS), tryptophan (Trp) and kynurenine (Kyn) were determined using Amlodipine (Aml) as internal standard (IS).
MATERIALS and METHODS:The analysis was carried out on a ACE-C18 (4.6mm × 50 mm, 5 µm) reversed-phase analytical column using gradient elution mode. For quantitative determination, Amlodipine was used as an internal standard. Detection was performed using multiple reaction monitoring in electrospray ionization mode at m/z 205.1→117.7 for tryptophan, m/z 209.1→146 and 93.9 for Kyn, m/z 409.2 → 294.1 for IS (Aml). Good linearity of an analyte to internal standard peak area ratios was seen in the concentration range 1.25–4000 ng/mL for tryptophan, 0.5–1600 ng/mL for kynurenine.
RESULTS:The method showed excellent linearity with regression coefficients 0.99 for Kyn and 0.996 for Trp. The limits of quantification (LOQ) were 0.55 ng/ml for Trp and 0.47 ng/ml for Kyn. %RSD for all analytes ranged from 0.3–3.4% for intra-day and 0.4–8.9% for inter-day experiments.
CONCLUSIONS:A simple LC-MS/MS method has been developed and validated for measuring Kyn and Trp by using an affordable and more easily available IS (Aml).
Keywords: Tryptophan, Kynurenine, Amlodipine, LC-MS/MS

P-003
Relation between the oligoclonal band presence and IgG index in multiple sclerosis

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OBJECTIVES:IgG index and Oligoclonal Band research are the prevalent tests in the diagnosis of Multiple Sclerosis disease (MS). The goal of this study is to evaluate the relation between the IgG index levels and Oligoclonal band (OCB) presense in cerebrospinal fluid (CSF).
MATERIALS and METHODS:Serum and CSF samples of 58 persons who applied to our laboratory for the test due to suspicion of MS, were included in the study. In the evaluation IgG index and simultaneously OCB in serum and CSF, were studied. Presence of OCB in CSF were evaluated by using isoelectric focus method. Albumin and IgG levels in serum and CSF were measured by immunoturbidimetri method.
RESULTS:In 24 (%41) samples OCB presense was seen. In 16 (%67) OCB (+) samples IgG index referance range limit was calculated as 0,7 and over. In 8(%33) OCB (+) samples IgG index was calculated as < 0,7. In 34 OCB (-) samples IgG Index was found 0,55 on average; in samples with IgG index under 0,55. In 21 (%36) samples of 58 IgG index was found at 0,55-0,70 range. In 8 (%38) of them OCB (+) was seen and this is also %33 of all OCB(+) samples.
CONCLUSIONS:In this study relation between IgG index and CSF OCB, that are common tests which are used in MS researches, was studied independently of cllinic value. When the index exceed 0,70 there was always connection, that OCB presence would be available between OCB analysis result and IgG index size.
Keywords: IgG index, Multiple Sclerosis, Oligoclonal Band

P-004
Comparison of the novel Access procalcitonin assay with Radiometer AQT 90 Flex

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OBJECTIVES:Procalcitonin (PCT) is considered the most useful biomarker for severe systemic inflammation, infection and sepsis although not totally specific for sepsis. Elevated PCT concentrations have a high positive predictive value for the diagnosis of sepsis, severe sepsis or septic shock (PCT >0.5 to >2 µg/L). Currently, there are several different assays available for PCT measurement. This study compared the performance of the AQT90Flex immunassay time resolved fluorometric technology with a new immunassay Access sysytem technology
MATERIALS and METHODS:148 EDTA-anticoagulated blood samples of hospitalised patients and outpatients for routine laboratory testing were included. The samples were analysed in duplicate for procalcitonin with RadiometerAQT90 Flex () and Access (Beckmann Coulter)
RESULTS:The Radiometer and the Access showed good correlation for the measurement of procalcitonin. The correlation coefficient (r) was 0,99 with (95% CI: of 99,1 to 99,5%). There are very small differences at very low concentrations which are of no clinical significance. A good correlation between the two methods was observed also in terms of clinical classification as indicated 0.5 ng/Ml. In particular, the percentage of concordance between the two assays using a cut off of 0.5 ng/mL is 98.4% (95% CI: 96.5– 98.5%).
CONCLUSIONS:In our study, the fully automated Access PCT agrees well with the Radiometer PCT and is suitable for early diagnosis of sepsis, severe bacterial infection and guiding antibiotic therapy
Keywords: procalcitonin

P-007
Evaluation of precision and bias of 10 analytes on Alinity c and i systems: A user perspective

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Verification of analytical performance of measurands becomes an essential requirement for the laboratories before proceeding to patients' samples testing. In our study we have verified the performance of ten analytes on Alinity ci systems against manufacturers' claims using CLSI EP15-A3 Guidelines. Manufacturer precision claims were obtained from analyte specific assay files(ASAF), and analyte specific product requirements documents(ASPR). After familiarization period, selected analytes were measured for 5 days in 3 replicates by using third party internal quality control(IQC) materials. Two and three levels of IQC material were used for Alinity c and i respectively. "Repeatability" and "Within laboratory imprecision" estimates were calculated and compared with manufacturer claims for precision evaluation. Varying levels of proficiency testing (PT) samples were used as reference material for bias estimation.Peer group means were used as target values(TV). Standard deviations from PT and precision results from our study were used to calculate standard errors(sec).Finally verifications intervals(VI) were calculated as VI=TV±(mxsec).All calculations performed by using R statistical software. An additional R script file is also created for reproducible calculations. For Alinity c, repeatability was between 0.3-2.4% coefficient of variation(CV) and Within laboratory imprecision was between 2.4-5.0% CV. For Alinity i, repeatability was between 1.7-5.3% CV and Within laboratory imprecision was between 5.3-7.7% CV. All analytes except creatinine and HbA1c had lower precision estimates than stated in ASAF. Creatinine and HbA1c had lower precision estimates than stated in ASPR.All analytes bias estimates were between VI. Our preliminary results show that our calculated precision and bias estimates are consistent with manufacturer claims.
Keywords: Abbott Alinity, user verification, CLSI EP15A-3, R

P-008
25-Hydroxy Vitamin D2 and 25-Hydroxy Vitamin D3 in lyophilized serum, UME CRM 1308

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OBJECTIVES:Vitamin D is a fat soluble vitamin in the form of vitamin D2 and vitamin D3. Measurement of vitamin D in serum is used in the investigation of bone health and emerging non skeletal conditions. 25-hydroxy vitamin D2 and 25-hydroxy vitamin D3 are the most common metabolites measured in human serum. Number of available Certified Reference Material (CRM) to be used in these measurements is very limited. In this study, the production, certification, homogeneity, stability and characterization of UME CRM 1308 "25-hydroxy vitamin D2 and 25-hydroxy vitamin D3 concentrations in lyophilized serum" is described.
MATERIALS and METHODS:UME CRM 1308 was prepared by adding 25-hydroxy vitamin D2 and 25-hydroxy vitamin D3 standards into the horse serum containing 25-hydroxy vitamin D3 endogenously. Horse serum was purchased from Biochrom AG (Germany) and pure standards were purchased from Sigma-Aldrich (USA). NIST SRM 2972 "25-Hydroxyvitamin D2 and D3 Calibration Solutions" and NIST SRM 972a Level 3 "Vitamin D Metabolites in Frozen Human Serum" were used for traceability. Isotope Dilution Liquid chromatography Mass Spectrometry (ID-LC-MS) was used for quantification.
RESULTS:The certified value is the mean of the ID-LCMS results, which is a primary method traceable to the SI. The certified value of 25-hydroxy vitamin D2 in human serum was 50 ng/g with an expanded uncertainty of 2.9 ng/g. The certified value for 25-hydroxy vitamin D3 was 48.8 ng/g with an expanded uncertainty of 2.6 ng/g.
CONCLUSIONS:CRM is used as a useful tool for proving traceability of measurement result and enhances measurement quality.
Keywords: ID-LC-MS, vitamin D

P-009
The importance of to determine their own SD values in medical laboratories

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OBJECTIVES:During internal quality control applications,in order to statistically evaluate the performance characteristics of a test measurement,the actual values for mean and SD should be determined in the laboratory.In this study,we calculated our own mean, SD, TAE values for 25 biochemistry tests in our laboratory.
MATERIALS and METHODS:2 levels of internal QC was performed once a day for 20 days in AU5800 (Beckman Coulter) instrument for 25 clinical chemistry tests.Using the QC data,mean and SD were calculated to compare with commercial QC material's values.For calculating TAE, %Bias values from external quality control data (Biorad) was used. TAE calculated by the formula proposed by the Ministry of Health.(%TAE=%Bias+1,65x%CV)
RESULTS:%CV values for 25 tests are between 0.4% and 3.7%.When our own SD and mean values are used to calculate TAE,all tests were within the appropriate range.The SDs recommended by the manufacturers were 2 to 12 times higher than our calculated SD values.When calculated SDs are used,the control and calibration should be performed more frequently as the false rejection rate increases.
CONCLUSIONS:Although it was recommended to use the calculated own SD values in textbooks and guides,no studies were found in the literature on this subject.In the study,we obtained results quite different from the commercially recommended SDs. If the manufacturer's suggested target and SD values are used,it will be difficult to notice errors. Although not cost-effective, each laboratory should use its own mean and SD values, as it is different from the manufacturer's.
Keywords: Mean, SD, %CV, TAe

P-010
Evaluation of performance characteristics of ELISA method for NGAL

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OBJECTIVES:Seeking for new useful biochemical markers in diagnosis of various diseases is a goal for many years. So far, NGAL is generally used as an early marker in kidney diseases, but recently, it was suggested to be one of the new early markers for diagnosis and prognosis of multiple sclerosis (MS). The aim of our study was to calculate the performance characteristics of NGAL measuring by ELISA method in patients with multiple sclerosis (MS).
MATERIALS and METHODS:MATERIAL-METHODS: Material for our study was plasma obtained from 30 healthy subjects (control group) and 55 subjects with diagnosed MS. NGAL was measured using ELISA method with commercial kits manufactured by Bioporto Diagnostics. Performance characteristics of interest were: sensitivity, specificity, positive predicative value, negative predicative value, accuracy, diagnostic odd ratios and were calculated using statistical program WinStat for Windows.
RESULTS:Our results have shown that the sensitivity and specificity of the test were 100% and 93% respectively, with 93% of positive predictive value and 41% of negative predictive value. The accuracy of the test was 74, 7%and the diagnostic odd ratio was 10,3.
CONCLUSIONS:We may conclude that ELISA method for measuring the concentrations of NGAL in patients with MS has satisfactory performance characteristics in discriminating healthy subjects from the patients correctly. Further studies are needed with a larger number of subjects.
Keywords: NGAL, performance characteristics, multiple sclerosis

P-011
Biological variation of newly developed red blood cell and reticulocyte parameters

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OBJECTIVES:Reliable biological variation data is needed for safe clinical application of laboratory tests. The aim of this study was to calculate biological variation of newly developed red blood cell parameters and reticulocyte indices used for diagnosis of anemia and monitoring of anemia treatment.
MATERIALS and METHODS:Blood samples were drawn from 30 healthy volunteers (20 female, 10 male) and analyzed using a Sysmex XN 3000 instrument during the 10 weeks period. Data were assessed in terms of normality, tendencies, outliers and variance homogeneity prior to applying coefficient of variance (CV)- analysis of variance (ANOVA) test. Sex-stratified within-individual (CVI) and between-individual (CVG) BV estimates of Hb, RBC, MCV, RBC-He (Hypo-He, Hyper-He, Micro R, Macro R), reticulocyte, reticulocyte-He (IFR, LFR, MFR and HFR) and Delta-He were determined.
RESULTS:For RBC parameters, with the exception of MCV, RBC-He, Hypo-He and Micro-R, and Delta-He there were significant differences between female and male CVI. However no differences were found for reticulocyte indices between both sexes.
CONCLUSIONS:New techniques and hematological parameters may reveal important information about functional integrity of bone marrow, diagnosis of anemia and monitoring anemia therapy. However, biological variation of these newly developed parameters should be considered in reporting and interpretation.
Keywords: Biological variation, anemia, red blood cell, reticulocyte.

P-012
Experimental study for determinate Risk-Based SQC procedure in our clinical laboratory by using six sigma

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OBJECTIVES:Six Sigma Methodology; is a quality management tool that focuses on process variables and provides information about process performance, and widely used in clinical laboratories. For risk-based SQC procedures, “The goal is to use a QC strategy that can detect change in performance reliably before the clinical quality requirement is exceeded while also minimizing the frequency of false rejections.” We aimed to design Risk-Based SQC plan for our laboratory and determine QC frequency by using six sigma.
MATERIALS and METHODS:Sigma metrics were calculated based on internal and external quality data by using (%TEa-%bias)/CV formula. Total allowable error (%TEa) was determinated analyte-based to the quality expectations of our laboratory in line with Milan hierarchy. The analyte-based workload of our laboratory was calculated and SQC run size nomogram was used for estimating the QC frequency.
RESULTS:In our study, Amylase, AST, ALT, D.Bilirubin, Triglycerid, Uric acid had >6 sigma value. In this context, they had longest QC frequency run size with >1000 patient sample by using 1:3s N:2 rules. Albumin, Chloride, Calcium, Creatinin, Sodium and Total Protein had <4 sigma value and the shortest QC frequency run with <45 patient sample by using 1:3s/2:2s/R:4s/4:1s N:4 rules.
CONCLUSIONS:It is important that clinical laboratories should have SQC plan for each analyte in which determine the levels, run size of control materials, and procedures for evaluating obtained control results. Risk-Based SQC procedure was found to be costly and difficult for analytes with low sigma values. In addition, tolerance limits should be harmonized for ensure an objective SQC plan.
Keywords: Six Sigma, Risk-Based SQC Schedule, Quality Management,

P-013
Effects of transportation time and seasonal temperature changes on routine coagulation tests

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OBJECTIVES:Pre-analytical issues in hemostasis testing are an important cause of diagnostic error and can lead to significant adverse clinical events. The aim of the present study was to investigate the impact of transport times and seasonal temperature changes on routine coagulation test results, that is, PT and aPTT.
MATERIALS and METHODS:Coagulation tests results were examined in the biochemistry laboratory of Antalya Training and Research Hospital from central and peripheral districts out-patients clinics. Results were divided into two groups to evaluate the seasonal changes effect (January, July) Transport times were less than 30 minutes for the central samples and 2-4 hours from the peripheral samples. Patients who applied for pre-operative investigation were included. Cardiovascular surgery patients were excluded. Coagulation tests were performed using the ACL TOP 500 analyzer.
RESULTS:According to the chi square test results with SPSS v21; there was not any significant difference between central and district outpatient activated partial thromboplastin time(aPTT) results. and also different season (January and July 2019) results. ($p>0,05$) On the contrary there was statistically significant difference between the prothrombin time(PT) results of two groups (central and district out-patient) due to chi square test, and also different season results (January and July 2019) $p <0.05$ According to the One Way ANOWA test results,there was no difference in the aPTT test for age groups. ($p>0,05$) There was a statistically significant difference in PT test. ($p <0.05$).
CONCLUSIONS:Preanalytical phase standardization in coagulation testing is critical to prevent unreliable results which might finally jeopardize the patient’s health.
Keywords: Preanalytical effects, Coagulation tests, temperatue, transportation

P-014
Agreement of hemoglobin and hematocrit values determined by co-oximetry and SLS hemoglobin: a retrospective study

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OBJECTIVES:Hemoglobin can be measured on a variety of devices using different methods. Blood gas devices have recently been widely used as point of care testing devices (POCT) in intensive care and emergency services. The reliability of the results obtained from these devices should be statistically tested. In this study, we aimed to see concordance of Co-oximetry and SLS Hemoglobin methods in the term of measuring Hemoglobin and calculated Hematocrit.
MATERIALS and METHODS:Between January 2019 and June 2019, 12049 patients who applied to the emergency department of Istanbul Training and Research Hospital were requested for complete blood count and venous blood gas analysis simultaneously. Samples were analyzed with Sysmex XN-1000 (SLS Hemoglobin) and Siemens Rapidlab-1265 (Co-oximetry). Passing Bablok and Bland Altman analysis were performed to show analytical methods concordance.
RESULTS:The correlation coefficient of both method for Hemoglobin and Hematocrit was 0.89 and 0.87, respectively ($P<0.0001$). Passing and Bablok regression analysis indicated that there was significant deviation from linearity ($p<0.01$). The Bland-Altman plot indicated that the two methods did not have good agreement for each tests. Bias % was calculated as 4% for Hgb and 1.1% for Hct. The Total Error was calculated as 5.8 % for Hgb. The calculated bias for Hct and calculated total error for Hgb was lower than that reported in online database by Westgard (6% for Hct, 7 % for Hgb).
CONCLUSIONS:Although each tests show significant deviation from linearity when comparing the two methods, blood gas devices could be used for Hgb measurements since the calculated bias remains acceptable.
Keywords: Blood Gas Devices, Co-oxymetry, SLS Hemoglobin

P-016
Effects of sampling time on routine coagulation tests in emergency service

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OBJECTIVES:Pre-analytical issues in hemostasis testing are an important cause of diagnostic error and can lead to significant adverse clinical events. The aim of the present study was to investigate the impact of sampling time on routine coagulation tests in emergency service, that is, PT and aPTT.
MATERIALS and METHODS:Coagulation tests results were analyzed in the biochemistry laboratory of Antalya Training and Research Hospital from emergency clinic. In January 2019, routine coagulation test results of patients admitted to the emergency department of Antalya Training and Research Hospital were examined retrospectively. The test results were divided into 6 groups at four hour intervals. Coagulation tests were performed using the ACL TOP 500 analyzer.
RESULTS:In this period, routine coagulation tests were performed in 1168 patients admitted to the emergency department. According to the one way ANOVA test results with SPSS v21; there was not any significant difference between the prothrombin time(PT) results of six sampling time groups($p>0,05$). On the contrary there was statistically significant difference between the activated partial thromboplastin time(aPTT) results of six groups ($p <0.05$).
CONCLUSIONS:Studies on circadian rhytms show that such variability can be observed with regard to many blood parameters, including parameters of hemostasis systems. Due to the nature of the emergency department must accept the patient for 24 hours. The reference value ranges for coagulation tests should be revised considering the circadian rhythm.
Keywords: Preanalytical effects, Coagulation tests, circadian rhythm, emergency department

P-017
Unnecessary test requests of HbA_2 in a university hospital

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OBJECTIVES:Laboratory physicians should be aware of unnecessary test requests because of cost effects. One of the most important contributors of this is unnecessary test requests. In this study, we aimed to reveal unnecessary test requests of haemoglobin A2 in a university hospital.
MATERIALS and METHODS:We retrieved Hemoglobin Variant analysis test request from laboratory information system between 01.01.2018- 04.07.2019. Hemoglobin Variant analyses were done with Tosoh G8 HPLC systems (Tokyo, Japan). Recurrent test requests were determined. All data were analyzed with Microsoft Excel 2010 Excel program.
RESULTS:There were 931 test request; 6 samples were rejected (3 inadequate volume and 3 inappropriate sample). 889 samples were analyzed with Tosoh G8 HPLC systems for variant hemoglobin. In 66 patient recurrent test requests were detected; in 59 patients there were two recurrent requests and in 7 patients there were three recurrent requests.
CONCLUSIONS:In the present study, we revealed only three test needed to repeat. In one sample patients' HbA2 test result 3.2% previously and his clinic is in accordance with iron deficiency anemia (IDA). We recommended IDA treatment and repeating HbA2 test recurrent request. After therapy of regular iron patient HbA2 result raised to 3.7 with microcytosis (MCV=68 fl) with normal iron condition. In the other two samples there were a decreasing in HbA2 because of hemolytic anemia so test request recurrence may be meaningful. Consequently, in 56 samples recurrent test request is unnecessary. Unnecessary test requests in HbA2 might be a problem in clinic laboratory and needed to solve.
Keywords: Unnecessary test, unnecessary test request, Hemoglobin A_2

P-018
Comparative analysis of the number of leukocytes in two different haematological modules

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OBJECTIVES:Counting of leukocytes (WBC) is an important part of routine laboratory tests. The aim of this study is to compare WBC values obtained by measurements performed on two hematology analyzers with assessment of their parallel use in laboratory work.
MATERIALS and METHODS:50 whole blood samples were included in the study. For the counting of WBC (103/μL), we used Sysmex XT-1800i reference module (fluorescent flow cytometry) and module Siemens Advia 2120 (flow cytometry). The accuracy and precision of the analyzers were checked. The results were statistically analysed in MedCalc software.
RESULTS:A shortened analytical evaluation has determined the satisfactory accuracy and precision of the analyzers. The lowest number of WBC measured on the Sysmex XT-1800i was 2.24, and the highest 12.25. The lowest number of WBC measured on the Siemens Advia 2120 was 2.3, and the highest 13.00. The Scatter diagram points to the diversity of data distribution. Bland Altman graph shows that almost all values were distributed within ± 1.96 SD. In Passing-Bablok regression analysis, when comparing the Sysmex XT-1800i with the Siemens Advia 2120, the following results were obtained for WBC $y = 0.0997170 + 0.966038 x$. Intercept a = 0.09972 (95% CI -0.04517 to 0.2750). Slopes b = 0.9660 (95% CI 0.9375 to 0.9897). Results for the slope indicate that there is a discrete proportional error, with no clinical significance. Cusum's linearity test estimates that there is no deviation in linearity ($P = 0.89$).
CONCLUSIONS:Both haematological modules can be used simultaneously in a routine laboratory practice.
Keywords: Leukocytes WBC, Flow cytometry, Hematology

P-020
Detection of deletional alpha thalassemia by multiplex PCR

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OBJECTIVES:Alpha thalassemia is a common genetic disorder that is characterized by deficient or absent synthesis of α-globin chains of the Hb molecule (HbA:2α2β). Alpha thalassemia usually result from deletions involving the α-globin genes, less commonly they are due to point mutations. Most α-thalassemia determinants are deletions involving one (α-thal-2: -α/αα) or both (α-thal-1: --/αα) α-globin genes on the same chromosome. A person with three α-genes (-α/αα) is not anemic but a heterozygote who inherits two functional α-genes (--/αα) has mild hypochromic microcytic anemia. Combinations of α-thal-1 and α-thal-2 determinants (--/-α) cause HbH disease. A patient who inherited a single α-globin gene (--/-α) has HbH disease with a chronic hemolytic anemia. Five different gene deletions [α-thal-1 (-17.4kb, -26.5kb, -20.5kb) and α-thal-2 (-3.7kb, -4.2kb)] were reported in Turkey.
MATERIALS and METHODS:Blood samples with EDTA as anticoagulant were taken for hematologic and hemoglobin analysis. A complete blood count was taken using a cell counter. HPLC have been used for both determination of hemoglobin variants and quantification of the Hb levels. Alpha globin gene deletions were detected by using one tube multiplex PCR.
RESULTS:In this study, alpha gene deletions of 81 carriers were detected by Multiplex PCR. Genotypes and hematological parameters of different α-thalassemia carriers were analyzed and 54 of them had one gene deletion (α-thal-2) and 27 of them had two alpha globin gene deletions (α-thal-1).
CONCLUSIONS:Distribution of alpha globin gene deletions were detected in Çukurova region.
This project was supported by Çukurova University Research Projects Unit (FDK-2019-11888).
Keywords: Alpha thalassemia, Gene deletion, HbH disease

P-021
Frequency of Silent Beta Globin Gene Mutations in Çukurova Region

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OBJECTIVES:The β-thalassemias are characterized by a quantitative deficiency of β-globin chains underlaid by a striking heterogeneity of molecular defects. There are two main varieties of β-thalassemia; β0-, and β+-thalassemia. The diagnostic feature of β-thalassemia is an elevated level of HbA2 in heterozygotes. There are, however, less common forms of β-thalassemia, which the HbA2 level is normal in heterozygotes. Broadly, they are classified into two varieties; type 1 called 'silent β-thalassemia' is no associated hematological changes, and the hematological findings of type 2 are typical of β-thalassemia trait with a raised HbA2. In this study, we aimed to investigate the incidence of silent beta thalassemia between 2008-2019 in Çukurova population.
MATERIALS and METHODS:Samples were obtained from Çukurova University Medical Biochemistry Department. DNA was extracted from whole blood. β-globin gene mutations were detected by Amplification Refractory Mutation System (ARMS) method, Restriction Fragment Length Polymorphism (RFLP) method and Deoxyribose Nucleic Acid (DNA) sequence analysis.
RESULTS:In our study, 3324 patients were retrospectively evaluated for hematologic data. 119 of 3224 patients were found to have normal hematological data. Silent β-thalassemia mutations were investigated in these patients. 93, 22, and 4 of totally 119 patients were detected as to be -30, -101, and CAP +1 mutations, respectively.
CONCLUSIONS:Our study showed that more common silent beta thalassemia mutations were -30 (T→A) in Çukurova Region. Despite normal hematological data, the possibility of silent β-thalassemia should not be excluded. As a result, it should be careful when evaluating individuals with normal hematological data for β-thalassemia.
Keywords: β-Thalassemia, Silent Beta Gene Mutation

P-022
Practice of an autoverification application

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OBJECTIVES:Autoverificaton systems are getting more prevalent day by day and becoming essential for clinical laboratories. The laboratories intending to install these systems should answer several questions and make several calculations. Unfortunately, there are limited scientific documents for evidence-based studying.
MATERIALS and METHODS:Our laboratory has the know-how that can be used as a starting point to calculate autoverification ranges and delta check limits; and to create algorithms for automatic release of results.. We have calculated autoverification limits with four different approachs: 1- Shih MC and friends's suggestion: Distribution intervals of patient data between %2 and %98 2- Feitosa MS and friends suggestion: A formula using the midpoint of reference range and linearity range 3- Limits from Li, Jiancheng and friends's article for thyroid hormones. 4- Analytical range at Troponin I and CK-MB Mass tests.
RESULTS:The acquired experience suggests the necessisity to generate a procedure to evaluate the specimens that have failed delta check evaluation. In our laboratory, over 20 thousand biochemistry tests are studied in a day and more than 600 of this tests fail at delta check evaluation. Unfortunately there is no enough manpower in clinical laboratories to examine those samples. In order to make this procedure realistic and feasible, it is necessary to reduce the rate of false positivity and thereby decrease the number of samples to be controlled.
CONCLUSIONS:Thanks to the experience gained and the new technical capabilities at hand to implement the medical information, autoverification is assumed to have potential for continuous developing without an end.
Keywords: Autoverification, Delta check, Limit check

P-023
***In Silico* analysis of missense mutations in the gene for human glutathione reductase**

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OBJECTIVES:At the molecular level, mutations are alterations in the nucleotide sequence of genes, resulting in variants that can be transmitted to the next generation. Mutations for which the clinical significance is currently unresolved are known as variants of uncertain significance (VUS). VUS generally involve missense mutations or in-frame deletions. As a part of the enzymatic antioxidant defense system, the homodimeric flavoprotein glutathione reductase (GR; EC 1.6.4.2) serves to regenerate glutathione (GSH) from glutathione disulfide (GSSG). Given the crucial role of GR in maintaining the body's GSH pools, hereditary GR deficiency is likely to have a dramatic impact on human health.
MATERIALS and METHODS:Here, we aim at predicting the structural consequences of clinically relevant missense mutations in the gene for human GR. The identities of missense mutations were retrieved from the Human Gene Mutation Database (HGMD) and ClinVar, and their effects on GR stability, flexibility and function were estimated using a diverse array of *in silico* prediction tools.
RESULTS:The sequence- and structure-based predictors reveal that nearly all of the missense mutations in question have the potential to affect local protein dynamics or enzyme catalysis. This allows for more accurate classification of the VUS into several different categories ranging from benign to pathogenic.
CONCLUSIONS:Overall, our work provides new insights into the 'molecular phenotypes' of hereditary GR deficiency and allow for the rational design of further *in vitro* and *ex vivo* studies.
Keywords: Missense mutations, variants of uncertain significance, glutathione reductase

P-025
Correlation between blood gas glucose parameter and biochemistry autoanalyzer glucose

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OBJECTIVES:Blood gas analysis is a vital diagnostic method on both clinical and emergency&intensive care patients.This study aimed to investigate the usability of glucose parameters measured on blood gas analyzer instead of biochemistry analyzers.
MATERIALS and METHODS:Blood gas and glucose parameters in biochemistry devices which was requested simultaneously from 23297 patients who applied to Izmir Katip Çelebi University Atatürk Training and Research Hospital were examined retrospectively between 01.07.2018-01.07.2019.Blood gas parameter was studied on Radiometer ABL800 Flex device and Abbott C16000 device was used for biochemistry parameter.Statistical analysis of the outcomes was performed in SPSS 21.0 program.The distribution of the groups was analyzed on Kolmogorov-Smirnov test.Paired t test was performed to get the significance of difference between biochemistry and blood gas glucose.Pearson test was used for correlation.
RESULTS:The groups were suitable for Gaussian distribution.The mean value of glucose on the automatic analyzer was found as 153.19 ± 90.11.Blood gas device glucose mean value was found as 150.26 ± 76.59.It was found that there was a statistically significant difference between biochemical glucose and blood gas glucose arithmetic mean($p<0.001$).Correlation coefficient was($R=0.936$ $p<0.001$) and the significant positive correlation was determined between outcomes.
CONCLUSIONS:According to the results of our study; although the difference was statistically significant, the values obtained were similar so as not to affect the clinical decision.Therefore,it is thought that glucose can be examined by means of blood gas analysis method until biochemical glucose parameters are resulted.This process will gain time to clinician especially on critical patients. Thus the possibility of early intervention will be increased accordingly.
Keywords: blood gas, correlation, glucose

P-027
Chemical analysis of the cyst fluid is an option to be kept in mind

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OBJECTIVES:Anamnesis, physical examination and radiological examination are generally sufficient to diagnose a cystic lesion in the neck. When needed, Fine Needle Aspiration Biopsy(FNAB) is a diagnostic tool in the management of neck masses.
MATERIALS and METHODS:61 years old male patient admitted to our hospital because of a lesion in the neck region. Physical examination followed by ultrasonography revealed a 25x17 mm subcutaneous cystic lesion located in superior anterior midline, strongly suggesting a thyroglossal duct cyst(TDC). The patient was unwilling for the excision of the lesion that a FNAB was performed. 4 cc opaque white material was sent to pathology and a portion of 1 cc was separated for chemical analysis for thyroglobulin and thyroid hormones.
RESULTS:Results showed that the cyst material contained 0,72 ng/mL thyroglobulin (1,6-50 ng/mL serum normal values), a free T4 level (0,55 ng/dL) lower than the patients serum FT4 value (1,11 ng/dL) and a free T3 level (3,13 pg/mL) higher than the patients serum FT3 value (2,74 pg/mL). Pathology report confirmed a benign lesion.
CONCLUSIONS:If a FNAB is performed with any reason, it should be kept in mind that the aspiration material is probably suitable for chemical analyses of various parameters. Thyroglobulin measurement is already recommended by the American and European Thyroid Association guidelines to diagnose cystic thyroid cancer metastases. The levels of tiroglobulin and thyroid hormones were supportive for a diagnosis of a TDC in this case although a final diagnosis is still absent because the lesion was not excised.
Keywords: Aspiration biopsy, Cystic lesion, Thyroglobulin, Hormones, Biochemistry

P-028
Myeloproliferative syndromes and their association with lymphoid neoplasms

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OBJECTIVES:The association of myeloproliferative syndromes with lymphoid neoplasia is very rare. There are about 52 cases, worldwide, where these two syndromes coexist within the same patient.
MATERIALS and METHODS:This abstract will present 2 clinical cases presented at the Haemetology Department which were initially presented as myeloproliferative syndrome, specifically: polycythemia vera, and subsequently lymphoid neoplasia, specifically: CLL, and Non-Hodgkin malignant lymphoma.
RESULTS:First CASE: Male, 51 years old, showing the following hematological parameters: WBC: 15000/m3, HGB: 14.5g/dL, RBC: 4.74x106/mm3, PLT: 175000/mm3.A myelogram and Immunophenotyping were performed on the patient, which were compatible with Polycythemia Vera, and no immunophenotipically pathogenic clone cells were identified. 2 months after the examination the patient showed up an adenopathy. A bone marrow biopsy was performed. The latter showed infiltration to the bone marrow of lymphomas with small CD 20 positive cells.
Second CASE: Male, 49 years old. Presented with a hematological framework compatible with the myeloproliferative syndrome: WBC: 20000/mm3, HGB: 17.2g/dl, RBC: 7.01x106/mm3, PLT: 966000/mm3.
A myelogram, Immunophenotyping and bone marrow biopsy were performed on the patient. The myelogram and the immunophenotyping resulted compatible with Polycethymia Vera, and no immunophenotipically pathogenic clone cells were identified.
Based on the results of the biopsy and immunohistochemistry the patient resulted in lymphoid infiltration by non-Hodgkin's malignant lymphoma.
CONCLUSIONS:The association of myeloproliferative syndromes with lymphoid neoplasia happens, more frequently in males. age of the affected is about 50 years. (in both cases presented), although in literature cases with this occurrence prevail in young ages.
Keywords: myeloproliferative syndromes, lymphoid neoplasia

P-029
Anticancer properties of novel BODIPY compound bearing pyridine groups

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OBJECTIVES:Colorectal cancer (CRC) is a common cancer type and treated with applications such as surgery, radiotherapy and chemotherapy. However, it is known that these methods have serious side effects. Therefore, alternative treatment strategies and new therapeutic molecules with less side-effect are needed. Photodynamic therapy (PDT) has emerged as a new method in the treatment of many cancer types. In recent years, boron-containing BODIPYs which are the photosensitizers, have been used in photodynamic therapy due to high absorption coefficient,high singlet oxygen yield, good solubility, low toxicity in dark. In this study, anticancer effects of water soluble pyridine group containing BODIPY compound (1a) were investigated against CRC.
MATERIALS and METHODS:Singlet oxygen quantum yield and CT-DNA binding effects of 1a were examined using UV-Vis spectroscopy. The pBR322 plasmid DNA photocleavage activities of 1a were investigated using agarose gel electrophoresis. The cytotoxic and phototoxic effects of 1a were tested against human colorectal (HCT-116) cell line using MTT assay for 24, 48 and 72 h.
RESULTS:Singlet oxygen quantum yield of 1a was found to be 0.30. The DNA binding studies showed that 1a bound to DNA with non-covalent interaction. 1a cleaved pBR322 plasmid DNA via singlet oxygen pathway upon irradiation. 1a showed remarkable phototoxic effect against HCT-116 in a concentration and time-dependent manner.
CONCLUSIONS:The results claimed that 1a had a potential anticancer agent for CRC. Further in vivo studies are required to clarify the therapeutic effect of 1a.
This study was supported by The Research Fund of Karadeniz Technical University (Grant no: 8134), Trabzon, Turkey.
Keywords: BODIPY; colorectal cancer; photocleavage.

P-030
Effects of somatostatin, curcumin and quercetin on the fatty acid profile of breast cancer cell membranes

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OBJECTIVES:Breast cancer is one of the most common cancer diagnosed in women in the world. Among the polyphenols; quercetin (Que), curcumin (Cur) have been reported to have strong potential to prevent breast cancer. However, so far, no comprehensive study has been performed to demonstrate anticarcinogenic effects of Cur, Que and their combinations with somatostatin on the fatty acid profile of breast cancer cell membranes.
MATERIALS and METHODS:The doses of somatostatin, curcumin and quercetin to be used in the incubations were determined by MTT test. For fatty acid analysis, membrane lipids were isolated, extracted, derivatized to methyl esters and characterized using Gas Chromatography in two breast cancer cells incubated with somatostatin, curcumin, quercetin, SST+Cur or SST+Que for 24 hours.
RESULTS:In MDA-MB231 cells, incubations with Cur, Que and SST+Que

induced the most significant membrane remodeling with elevation of stearic acid, and diminution of omega-6 linoleic, arachidonicacids, omega-3 acids. In MCF7 cells, omega-6 linoleic acid in cells incubated with SST+Que, Que increased and omega-3 fatty acids in cells incubated with SST+Cur compared to SST decreased, and significant increases in docosapentaenoic acid levels were found in cells incubated with SST+Que compared to the control cells.
CONCLUSIONS:Based on our findings, lipid isomerization in breast cancer cells has been shown to change in response to Somatostatin, Cur, Que and their combinations. The results of lipidome analysis highlighted the role of SST+Cur and SST+Que induced fatty acid membrane remodeling, and suggest potential of lipid-based strategies for influencing cell response in breast cancer cells.
This study was supported and funded by TUBITAK (Project number: 217S253) and Akdeniz University Research Funds (TDK-2017-2096).
Keywords: Somatostatin; curcumin; quercetin; breast cancer; membrane fatty acid profile

P-031
Investigation of the effect of twist1 suppression on miRNA level in MDA-MB 231 breast cancer cells

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OBJECTIVES:MDA-MB 231, which has a more aggressive structure compared to other breast cancer cell lines, is metastatic and angiogenic cells. Metastasis and angiogenesis are regulated by transcription factors such as the Twist1 gene. Twist1 is a transcription factor (TF) that can bind specific DNA regions and control the activity of genes. Twist1 is effective in various differentiation processes of healthy embryos as a positive or negative regulator in the cell cycle. In studies, Twist1 gene has been shown to result in a poor prognosis in patients with protein re-expression after embryonic period. Twist1 also regulates the expression of micro-RNA (miRNA) genes associated with cancer. Many studies have shown that these small molecules are critical in cellular mechanisms such as metastasis, angiogenesis and apoptozis. In this study, the effect of silencing Twist1 TF gene which active in MDA-MB 231 breast cancer cell was affected by expression levels of miRNAs.
MATERIALS and METHODS:In the MDA-MB 231 cells, the regulatory Twist1 gene was suppressed by the anti-sense Twist1 vector transfection. Differences in miRNA expression levels were analyzed by Real Time PCR analysis.
RESULTS:As a 43 miRNA results examined in the study, it was found that miR-1-1 and miR-210-3p expressions were upregulated and miR-193b-3p, miR-181b-5p and miR-148a-3p expressions were downregulated.
CONCLUSIONS:The expression levels of some miRNAs associated with invasion, metastasis and apoptozis were changed by silencing Twist1 gene expression. It was concluded that silencing the Twist1 gene may effect invasion, metastasis and apoptozis in breast cancer.
Keywords: MDA-MB 231; miRNA; twist1

P-032
Molecular and cellular biofunctional analyses in Turkish patients with invasive bladder carcinoma

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OBJECTIVES: Bladder carcinoma (BC) is the most frequently seen urinary system malignancy among men all over the world. In this study, we aimed to identify specific gene-pathway relationships that could play fundamental role in the progression of bladder carcinogenesis.
MATERIALS and METHODS:Samples (n=12) of high-grade invasive bladder cancer patients and their non-tumoural paired controls (n=12) were collected at the Department of Urology of Istanbul Faculty of Medicine after getting ethical permission from Istanbul University. The genome wide expression levels of high grade BC patients and paired controls were profiled following to RNA isolation and hybridization steps. Ingenuity Pathway Systems (IPA), iPathway Guide and Cytoscape softwares were used to determine statistically significant genes, networks, biological pathways between tumor and control groups.
RESULTS:The most statistically significant biofunctions were post-translational modification, protein degradation, cell death and cell survival, cellular movement and intercellular signalling. These findings were supplied us understanding which molecular reactions were involved in bladder carcinogenesis. In regards to these results, we also identified the top canonical pathways that were included in the development of BC. The data showed that collagen degradation, activation of matrix metalloproteinases (MMPs), degradation of the extracellular matrix (ECM), inflammatuar response and BC signalling were the most important pathways in bladder carcinogenesis, as expected.
CONCLUSIONS:We showed that biological reactions including degradation of collagen and ECM as well as MMP activation reactions were found the most statistically significant pathways in BC. It was also determined that inflammation and cytokine signalling could be related with the progression of bladder carcinogenesis.
Keywords: Invasive Bladder Carcinoma, Biological Pathways, Matrix Metalloproteinases, Degradation of the Extracellular Matrix

P-033
Investigation of antioxidant and cytotoxic properties of Amphoricarpos Praedictus

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OBJECTIVES:Amphoricarpos Praedictus (Testiotu) is a member of the Asteraceae family. Many Asteraceae species are rich in secondary metabolites such as phenolics, sesquiterpenes and lactones. Asteraceae species are known as medicinal plants with antioxidant effect, and antimicrobial activities. Literature is limited about the biological activity of Amphoricarpos species.The aim of this study was to determine the antioxidant and cytotoxic properties of Amphoricarpos Praedictus.
MATERIALS and METHODS:The present study assessed total phenolic and flavonoid content, reducing antioxidant power, radical scavenging activity of Amphoricarpos Praedictus by using spectrophotometric methods. The cytotoxic effect of Amphoricarpos Praedictus on a normal human lung fibroblast (WI-38) cell line was assessed using the XTT assay.
RESULTS:Accordingly, the results of the Amphoricarpos Praedictus exhibited higher radical scavenging activity, and selective cytotoxic effect on WI-38 cells. These results showed that the Amphoricarpos Praedictus has power antioxidant properties and selective cytotoxic effect.
CONCLUSIONS:Thus, Amphoricarpos Praedictus appear to be a promosing source of new anticancer agent.
Keywords: Amphoricarpos Praedictus, Antioxidant activity, Cytotoxic Activity

P-034

Some critical gene genotypes belongs to coinhibitory and costimulatory signals ın the tumor microenvironment in laryngeal cancer

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OBJECTIVES:T cell regulated signals play an important role in maintaining peripheral tolerance immune homeostasis. Studies have also reported that the coinhibitory and costimulatory signals interact with each other like as PD-1 / PDL-1 interaction triggered cd28 inactivation in the suppression of T cell activation. In this study, we aim to investigate PD-1 (rs2227981), PDL-1 (rs2890658), CD28 (rs2267966) and CD27 (rs3116496) genotypes in laryngeal cancer (LC) patients.
MATERIALS and METHODS:We examined PD-1 (rs2227981), PDL-1 (rs2890658), CD28 (rs2267966) and CD27 (rs3116496) polymorphisms in 132 subjects (57 subjects with LC and 75 controls) by using PCR-RFLP.
RESULTS:We found an increased frequency of PDL-1 AA/CC genotypes in laryngeal cancer patients (p=0.04) than controls but not for PD-1 genotypes. There was a tendency toward a higher frequency of CD 28 T allele and CT genotype,respectively(p=0.034, Odds ratio (OR): 1,180; 95 %CI1,019-1,366; p=0.001, odds ratio (OR), 2,538; 95% CI 1,470-4,380). CD27 genotype results showed a higher incidence of A allele in patients versus controls, p=0.001, OR: 1.228; 95 %CI 1,091-1,382).The frequency of AT genotype was found to be increased in laryngeal cancer patients and this value was statistically significant p=0.002, OR, 1,888; 95% CI 1,258-2,833. We also found significant relationships between these genotypes and patient's clinical and histopathological findings, perineural invasion, the presence of reflux.
CONCLUSIONS: Our results suggest that CD27, PD1 and PDL1, especially CD28 are thought to be important candidates implicating some changes affecting this mechanism. These molecular markers may use to be target molecules for identifying subjects, better prognosis and response to treatments.
Keywords: Laryngeal Cancer, İmmune Checkpoints, Molecular Biomarkers, Carcinogenesis, Polymorphism

P-035

Investigation of in vitro and in vivo therapotic effect of curcumin and 5-FU on colon cancer

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OBJECTIVES:This study aimed to investigate the cytotoxic, genotoxic, apoptotic, and anti-cancer effects of curcumin – the active ingredient of turmeric – and 5-Fluorouracil in combination against in vitro and in vivo colon cancer while illuminating possible action mechanisms.
MATERIALS and METHODS:Initially, Luciferase transfection was performed in LoVo colon cancer cells to which curcumin, 5-FU, and combinations of different concentrations were given, and they were incubated for 24 hours. Cytotoxicity, genotoxicity, apoptosis, intracellular glutathione level, mitochondrial membrane potential are determined. Transfected LoVo cells have been injected subcutaneously to nude mice for the following: control, curcumin, 5-FU, and combined. 3 weeks of treatment with curcumin, 5-FU, and combined therapy have been initiated 3 weeks after the injection. At the end of this period, tumor size was measured with the IVIS device and caliper.
RESULTS:Compared to the monotherapy with curcumin and 5-FU on colon cancer, combined treatment has been found in low doses to increase cytotoxicity, DNA damage, apoptosis and intracellular reactive oxygen species in the cell culture studies, while decreasing mitochondrial membrane potential and glutathione levels. Also, the expression of apoptotic proteins increased while the anti-apoptotic protein expression decreased. Combination therapy was found to be more effective than mono therapies in vivo colon cancer, which was formed by the xenographic method. While tumor size decreased.
CONCLUSIONS:According to the data obtained through this study, in colon cancer curcumin has been found to increase the anti-tumor effects of the normal therapy of 5-FU in vitro and in vivo.
Keywords: curcumin, colon cancer, 5-FU, IVIS

P-036

Clinical significance of soluble DCR3 in breast cancer patients before and after radiotherapy

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OBJECTIVES:Breast cancer is one of the most important cancer type in the world. Radiotherapy plays an important role in the treatment of non-metastatic breast cancer. The DcR3 family, known as tumor necrosis factor receptor superfamily member (TNFRSF6B). It has emerged important regulators of various biochemical events as it has roles in various cancer and several inflammatory tissues. The aim of this study was to determine the effect of radiotherapy on soluble DcR3 protein, which has not been yet clarified as a tumor suppressor or promoter molecule.
MATERIALS and METHODS:22 women with non-metastatic breast cancer enrolled to the study. Blood samples were taken just before and after radiotherapy, soluble DcR3 proteins levels were determined with ELISA kit. Wilcoxon test was used for statistical analysis.
RESULTS:Soluble DcR3 protein level was significantly found to be decreased after radiotherapy treatment (p<0.011).
CONCLUSIONS:Our study demonstrated that soluble DcR3 could be considered as a novel follow up parameter for the treatment of breast cancer malignancy. In other words, modulation seen at the soluble DcR3 protein level suggests that it may also provide a new strategy for breast cancer treatment. This work is the part of project entitled 'Evaluation of the effect of radiotherapy on some biochemical parameters in breast cancer patients' supported by the grand from Erciyes University (Grand no:TYL-2019-8672).
Keywords: DcR3, Breast Cancer, Radiotherapy

P-037

Low dose Bisphenol A and Fulvestrant ıncrease the proliferation and migration of hepatocellular carcinoma increase

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OBJECTIVES:Fulvestrant (ICI 182 780), a selective estrogen receptor inhibitor, has been used in treating patients with hormone-sensitive breast cancer. Bisphenol A (BPA) has been considered as an endocrine disrupting chemical. In this study, we examined whether the effect of low dose BPA on fulvestrant treatment can lead to the proliferation and migration of HepG2 human hepatoma cells which may induce metastasis.
MATERIALS and METHODS:Human hepatocellular carcinoma cells (ATCC) were treated with certain concentrations BPA, ICI and the combination of ICI with BPA. MTT assay was conducted to examine the effect of BPA, ICI and BPA+ICI on cell proliferation of HepG2 cells. Effects on cell migration were examined by wound healing assay and results were analysed by Image J software. Additionally, the expression of N-cadherin were detected by N

Cadherin-Enzyme-Linked ImmunoSorbent Assay.
RESULTS:Low dose BPA and BPA+ICI significantly increased cell viability of HepG2 cells compared to vehicle control. Their effects on motility of HepG2 cells were measured by the use of wound healing test and as a result significantly increased wound closure was determined as compared to the control group. BPA also stimulated the migration of HepG2 cells. BPA+ICI increased N-cadherin expression which might be the indicator of epithelial to mesenchymal transitions.
CONCLUSIONS:According to these results, it was suggested that BPA and Fulvestrant may induce the proliferation and migration in HepG2 cells
Keywords: Bisphenol A, fulvestrant, HepG2

P-038
Regulation of intracellular ROS level under different stress conditions in HepG2 Cells

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OBJECTIVES:One of the main features of cancer cells is a persistent pro-oxidative state that leads to intrinsic oxidative stress. Additionally, the inflammatory condition of malignant tumors continually exposes cancer cells to reactive oxygen species (ROS) and the activation of the antioxidant defense system. Quercetin is an antioxidant flavonoid known to induce cell cycle arrest and apoptosis of hepatocellular carcinoma cells. Activation of several signaling pathways has been implicated in the pathogenesis, i.e. ROS which can trigger oxidative damage of biomolecules. The objective of the present study was to determine the effect of treatment with hydrogen peroxide and quercetin on HepG2 cells.
MATERIALS and METHODS:Effects of different stress conditions were evaluated. For this purpose, cells were cultured under two different stress conditions. Cell viability/apoptosis/cell cycle, proteasome and antioxidant enzyme activities were detected.
RESULTS:Hydrogen peroxide and quercetin resulted in decrease cell viability of HepG2 cells in 30 minutes. The percentage of total apoptosis were 9.66 and 10.93 for H2O2 and quercetin, respectively at 50 µM concentrations and 16.45 and 18.78 for H2O2 and quercetin, respectively at 200 µM concentrations. Quercetin decreased proteasome activity significantly. Quercetin also influenced cell cycle distribution and significantly decreased G0/G1 ratio.
CONCLUSIONS:Our findings demonstrate the pleiotropic effects of quercetin on liver cancer cells and open the possibility of utilizing it as a chemo-preventive agent in hepatocellular carcinoma.
This work was supported by The Scientific and Technological Research Council of Turkey TUBITAK (Project no: 21S963).
Keywords: HepG2 cells, hydrogen peroxide, quercetin

P-039
Antioxidant effect of static magnetic field on breast cancer cell line

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OBJECTIVES:Oxidative stress is thought to take part in the etiopathogenesis of many systemic diseases, including cancer. SMF plays a critical role in activating and/ or alternating the molecular mechanisms in cancer cells. The aim of this study was to find out the antioxidant effect of static magnetic field on breast cancer MCF-7 cell line.
MATERIALS and METHODS:Oxidative stress determinations were performed by total antioxidant status (TAS) (Relassay), total oxidant status (TOS) (Relassay) in MCF-7 cell line. Oxidative stress index (OSI) was calculated by TOS/TAS ratio. Statistical analyzes were evaluated by SPSS programme.
RESULTS:TAS levels were found to be significantly increased in SMF exposed group compared to controls [(0.201 ± 0.003 vs 0.183 ± 0.002 mmol Trolox Equiv./L, p<0.001)]. OSI levels were significantly lower compared to controls [(0.311 ± 0.005 vs 1.00 ± 0.117, p<0.001)].
CONCLUSIONS:Taken together, our results suggest that exposure of SMF on MCF-7 cell lines diminished oxidative stress parameters. According to these results, SMF administration can increase the antioxidant effect; this may offer a protective strategy for cancer therapy.
Keywords: TAS; TOS; MCF-7; Static Magnetic Field.

P-040
3-Aminopropyltriethoxysilane coated magnetite for using as support to reduce toxic effects of idarubicin on HL60 cell line

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OBJECTIVES:Cancer drugs are one of the most studied topics, especially since their half-life is very short, the disintegration products are toxic and they cannot distinguish between healthy or diseased cells. This increases the number of studies on immobilization by binding to many organic or inorganic support materials through intermolecular interactions or covalent bonds. The aim of this study was to immobilize idarubicin via imine band to 3-Aminopropyltriethoxysilane (3APTES) coated magnetite, to prepare a cancer drug with stability and low toxicity levels.
MATERIALS and METHODS:IDA was immobilized to 3-APTES coated magnetite and its activity in HL-60 cell line was studied. Synthesized materials were characterized by spectroscopic devices. IDA immobilized magnetite was administered to HL60 cell line with various doses, ATP and MTT cell viability analyzes were studied and compared to free IDA. In this study, idarubicin was immobilized to an amine group for the first time.
RESULTS:IC50 value of immobilized IDA was 4-folds lower than that of free IDA in HL60 cell line according to in-vitro cytotoxicity tests. Furthermore, idarubicin binding amount was calculated as 0.2 g/100 g magnetite/3APTES.
CONCLUSIONS:The results of this study showed that magnetite-induced idarubicin is effective in eliminating cancer cells even at doses 4 times lower. By applying this method in the clinic, patients will experience less toxic exposure. Using this structure for the first time and giving better results than free idarubicin will provide a new approach to cancer.
This study was supported by TUBITAK BIDEB 2218.
Keywords: Magnetite, HL60 cell line, 3-Aminopropyltriethoxysilane, idarubicin

P-043
Comparison of immunoassay methods (CMIA and ECLIA) for determination of tumor markers HE4 and CA 125

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OBJECTIVES:In our study, we investigate the performance of the Cobas e 601 HE4 and CA 125 compared with Architect and SR 2000.
MATERIALS and METHODS:The investigation included 200 serum samples that were investigated using Cobas e 601 (ECLIA) and Architect and SR 2000 (CMIA).
RESULTS:Using CMIA, the coefficients of variation (CVs) varied from 2.13 % to 4.16 % for CA 125 and from 1.10 % to 4.60% for HE4, and reproducibility from 2.60% to 5.20% for CA 125 and from 1.70% to 4.90 % for HE4. Using Cobas ECLIA, the CVs varied from 0.55 % to 2.09 % for CA 125 and from 0.36 % to 2.30 % for HE4, and reproducibility from 1.0 % to 3.50 % for CA 125 and from 070 % to 4.05 % for HE4. The CMIA and ECLIA regression equation for CA 125 was y (ARCHITECT) = 1.675 + 1.027 x(Cobas) and have intercept

(95% CI 0.418 to2.932) and slope (95% CI: 0.979 to 1.076). The regression equation between CMIA and ECLIA for HE4 was y (ARCHITECT) = 4.330 + 1.502 x(Cobas) and have intercept (95% CI -8.461 to 17.12) and slope (95% CI: 0.924 to 1.373). A high agreement was found between the two immunoassays for determination HE4 and CA 125.
CONCLUSIONS:The various immunoassay techniques using different monoclonal antibodies and methods of detection which leads to different results. The tumor markers CA 125 ad HE4 should be determined if it is possible in only one method.
Keywords: CMIA, ECLIA, HE4 and CA 125

P-045
Therapeutic potential of targeting miR-196a through proliferation and clonogenicity in human PDAC cells

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OBJECTIVES:OBJECTIVES: Pancreatic cancer is known to be one of the most lethal human cancers with 2-3% 5-year survival rate due to strong invasive and metastatic properties and high recurrence capacity. Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer with 95% of all pancreatic cancers. Therefore, the novel therapeutic agents urgently need to be developed for these patients. However, it is only possible when molecular mechanisms related to PDAC are investigated in detail. To be able to develop the new therapeutic approaches for PDAC, we aimed to investigate the role of miR-196a in cell proliferation and clonogenicity in Panc-1 and MiaPaCa-2 PDAC cell lines. The therapeutic potential of targeting miR196a was investigated using specific inhibitor because of high oncogenic properties of miR-196a in cancers.
MATERIALS and METHODS:MATERIALS-METHODS: For this purpose, MTS and cell colony formation assays were performed for the evaluation of cell proliferation and clonogenicity, respectively, followed by transfection of Panc-1 and MiaPaCa-2 cells with 50 nM negative miRNA or miR-196a inhibitor for 72 h under regular culture condition.
RESULTS:RESULTS: Our data clearly shown that miR-196a inhibitor decreased cell proliferation in both Panc-1 and MiaPaCa-2 cells as 38.6% and 42.1%, respectively, compared to negative miRNA. Additionally, cell clonogenicity were decreased when the both cells transfected with miR-196a inhibitor.
CONCLUSIONS:CONCLUSIONS: According our results, the downregulation of miR-196a might be the new therapeutic approach for PDAC. We believe that these data will help to guide both our further investigations and other researcher in this field.
Keywords: pancreatic cancer, PDAC, miRNA, miR-196a, cell proliferation

P-048
Curcumin inhibits the cell survival and induces apoptosis of human colorectal cancer HCT116 cells

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OBJECTIVES:Curcumin has been used as a medicinal plant and a food additive and has many different biological activities such as anti-inflammatory, anti tumor, anti-oxidant effects and induces apoptosis and inhibits cell proliferation in different cells. We aimed to investigate the effect of curcumin on HCT-116 colorectal cancer cell line.
MATERIALS and METHODS:Cells were treated with different concentration of curcumin for 24 h. Cell survival rate and cell migration was measured by MTT assay and scratch assay, respectively. Apoptosis was monitored by Acridine Orange and Propidium Iodide double staining. The expression level of MMP-9, P53 and Caspase 3 was analyzed by RT-PCR.
RESULTS:Curcumin decreased cell survival and migration rate after 24 h compared to control ($p<0.0001$). Curcumin down-regulated the expression level of MMP-9 and Caspase 3 and up-regulated the expression level of P53 ($p< 0.0001$). The highest percentage of apoptosis observed at concentration of 5 µM curcumin.
CONCLUSIONS:These results demonstrated that curcumin inhibits HCT-116 cells survival, migration and invasion and also curcumin could induce apoptosis through modulating the expression of apoptotic genes.
Keywords: cells survival, proliferation, apoptosis, invasion, migration

P-049
High levels of circulating undercarboxylated matrix Gla-protein found in patients with cardiovascular diseases

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OBJECTIVES:The aim of the current study was to assess whether serum undercarboxylated matrix Gla-protein (ucMGP) levels are associated with coronary artery calcium score (CACS) in a moderate-to-high risk patients without and with cardiovascular diseases (CVD).
MATERIALS and METHODS:A total of 67 patients (48 females and 19 males), mean age 69.4±11.9 years) who visited the Cardiology Clinics at the University Hospital of Varna between October 2018 - July 2019 were enrolled in the study. Moderate- and high-risk patients without CVD (n=33) served as controls. Patients with paroxismal and persistant atrial fibrillation and those with heart failure and sinus rhythm with ejection fraction >40% repesented the CVD group. All participants underwent a multislice computed tomography examination. Fasting venous blood was drawn for laboratory tests. The measurements of ucMGP were performed using a competitive mono-antibody ELISA kit (Cusabio Technology LLC, USA). The routine biochemical parameters (lipid profile and uric acid) were assessed on automated biochemical analyzer. Standard statistical methods (descriptive statistics, one way ANOVA, Spearman correlation analysis) were applied.
RESULTS:Increased ucMGP levels by 24.3% were found in the CVD group when compared to the controls (4.489±1.081µg/ml vs 3.612±0.508µg/ml). Significant positive correlation was established between ucMGP levels, CACS (r=0.29), total cholesterol (r=0.32), and uric acid levels (r=0.72).
CONCLUSIONS:This preliminary study shows that high ucMGP concentrations are associated with higher CACS in CVD patients. In this regard, our results are consistent with the findings of other studies and contribute to the hypothesis that ucMGP plays a pathophysiologic role in vascular calcification.
Keywords: undercarboxylated matrix Gla-protein, coronary artery calcium score, cardiovascular diseases

P-050
Evaluation of copeptin, ghrelin and proBNP levels in patients with metabolic syndrome

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OBJECTIVES:Metabolic syndrome (MetS) is characterized by the coexistence of systemic diseases such as abdominal obesity, insulin resistance, hypertension, hyperlipidemia, that increases the risk of diabetes and cardiovascular diseases. Arginine vasopressin (AVP) may be implicated in MetS by altering pituitary ACTH release, liver glycogenolysis, and secretion of glucagon and insulin. Copeptin is considered as an useful surrogate marker for AVP. It has been suggested that ghrelin with multiple functions including modulating appetite, control of energy metabolism and vascular function, is involved in the development of MetS. The pro-B-type natriuretic peptide (proBNP) has also been proposed as a promising cardiac biomarker for heart function. The aim of this study was to evaluate the relationship between MetS and three different bio markers that related to metabolism and cardiovascular system.
MATERIALS and METHODS:The present study enrolled a total of 44 patients with MetS and 30 healthy subjects. Serum copeptin and ghrelin levels were determined using the ELISA technique. The serum levels of proBNP were measured by using chemiluminescence method. The biochemical parameters including blood glucose and insulin levels, lipid profiles were also measured.
RESULTS:Copeptin and ghrelin levels were significantly lower in patients with MetS than controls. ProBNP levels were significantly higher in patients than in controls.
CONCLUSIONS:Depending on our outcomes it can be postulated that ghrelin, copeptin and proBNP may be associated with components of MetS and involved in pathogenesis of MetS. Therefore, these parameters are thought to be a guide in the follow-up of risky patients.
Keywords: Copeptin, ghrelin, metabolic syndrome, proBNP

P-051
Plasma levels of ApoB, ApoA1, and Apo B/A1 ratio are associated with coronary artery disease

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OBJECTIVES:Apolipoproteins play an important role in lipid metabolism. Dyslipidemia is well known risk factor for CAD, thus apolipoproteins are considered as risk factors for coronary artery disease, too. The aim of this study was to evaluate the association of higher apolipoproteins levels, ApoB/A1 ratio and coronary artery disease (CAD).
MATERIALS and METHODS:ApoA1 and Apo B were determined by immunonephelometric methods in plasma of 119 CAD patients and 182 healthy subjects; Apo B/A1 ratio was calculated mathematically.
RESULTS:Patients with CAD compared to healthy subjects had statistically significant higher levels serum levels of triglycerides (2.54 ± 0.79 vs. 1.32 ± 0.41, p<0.001), total cholesterol (5.49 ± 1.33vs. 4.65 ± 0.99; p<0.0001), LDL cholesterol (3.55 ± 1.02 vs. 2.79 ± 0.96;p<0.001), Apo B (1.27 ± 0.41 vs.0.78 ± 0.25, p<0.0001), and Apo B/A1 ratio (1.20 ± 0.37 vs. 0.65 ± 0.21 p<0.0001); decreased levels of ApoA1 (1.073 ± 0.21 vs. 1.19 ± 0.19, p< 0.002) and HDL cholesterol (0.89 ± 0.24 vs. 1.22 ± 0.27, p<0.0003) were found in CAD patients compared to control group
CONCLUSIONS:The results indicate that dyslipidemia and Apo B levels, apoB/A1 ratio and decreased apoA1 are associated with CAD.
Keywords: CAD, apolipoproteins, dyslipidemia

P-053
Hematological indices as biomarkers of early cardiac adverse events after acute myocardial infarction

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OBJECTIVES:This study's objective is to explore the value of hematological indices as biomarkers of major adverse cardiovascular events during the hospitalization after acute myocardial infarction (AMI).
MATERIALS and METHODS:230 patients with AMI were enrolled in this study. Venous blood was collected at admission. Total white blood cell (WBC), neutrophil, lymphocyte, monocyte, platelet (PLT) counts, MPV, PDW and RDW were calculated using an automated blood cell-counter. Neutrophil-to-lymphocyte ratio (NLR) was computed from the absolute values of neutrophils and lymphocytes. Patients were categorized in groups according to the occurrence or not of heart failure, rhythm disorders and survival. Statistical analysis was performed using SPSS/IBM. P-value<0.05 was considered statistically significant.
RESULTS:AMI patients (mean age 68+/-12, 73.5% males) had an average WBC count of 9611/mm3 despite their in-hospital outcomes. Major adverse cardiovascular events happened in 99 patients (43%). WBC, Absolute Neutrophil Count (ANC), NLR and Monocyte Count (P<0.001), PLT (p=0.046), MPV (p=0.003), PDW (p=0.042) were significantly higher in patients who developed heart failure compared to those who didn't. Cut-off values ANC 7120/mm3 and NLR 4.215 show high sensitivity and sensibility for heart failure. Nonsurvivors had higher WBC, ANC, MPV, PDW, RDW (p<0.001), NLR (p=0.005) at admission. Old age, female gender and ST-segment elevation (STEMI) had significantly higher mortality. Heart rhythm disorders were better predicted by higher WBC (p=0.019) and ANC (p=0.036).
CONCLUSIONS:Hematological indices are useful and cost-effective cardiovascular biomarkers for the occurrence of heart failure, rhythm disorders and death during the hospitalization after AMI, potentially impacting the clinical prognosis and management.
Keywords: hematological indices, cardiovascular biomarkers

P-055
Correlation between Troponin I and complexity of coronary lesions in patients with acute coronary syndrome

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OBJECTIVES:The aim of our study is to explore the correlation of Troponin I (TPI) with the complexity of coronary lesions in patients with Acute Coronary Syndrome (ACS) with evaluation according SYNTAX score, and to evaluate the correlation of Troponin I (TPI) with TIMI and GRACE clinical scores, as predictors of future cardiovascular events.
MATERIALS and METHODS:We studied a group of 107 patients: 31 females and 76 males with ACS who underwent coronary angiography. TPI is measured by chemiluminescent immunometric assay. Statistical analysis of clinical, laboratory and coronary angiographic data was performed by the SPSS 22 program.
RESULTS:The average age of the studied population is 62.71±10.4. Most of patients were in Killip1 (87.9%) also 26 % of patients had STEMI, 52.1% Non STEMI, and 21.9% unstable angina. TPI levels are significantly higher in group with stenosis ≥ 50% vs. group without stenosis < 50% (77.33 ng/ml vs. 2.5 ng/ml, p = 0.000). Positive correlation was found between levels of TPI and Syntax score (r = 0.416, p = 0.00), TIMI score (r = 0.514, p = 0.00) and GRACE scores (r = 0.509, p = 0.00). TPI levels between IAM STEMI and IAM Non STEMI was not statistically significant (122.3±42 ng/ml vs 100.5±32 ng/ml, p = 0.0678).
CONCLUSIONS:TPI is a reliable biomarker in evaluation of the complexity of coronary lesions, we found a positive correlation between TPI levels and Syntax score, also TPI has a positive correlation with TIMI an GRACE clinical score, suggesting a higher risk of future cardiovascular events.
Keywords: Troponin I, Acute coronary syndrome

P-058
The relationship between the degree of stable angina pectoris and serum pentraxin level

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OBJECTIVES:Latter studies demonstrate that inflamation has a key role in the whole atherosclerotic process; onset and progression. One of the atherosclerosis spesific inflamattory indicator is pentraxin 3 which is synthesised from cells, found in atherosclerotic origin, such as endotel, macrophages, smooth muscle cells.PTX-3 is one of the member of pentraxin family such as hs-CRP and a component of the natural immunity. In our study, we evaluated the serum PTX-3 of patients who have diagnosis of the "stabile angina pectoris" and whom coronary obstruction degree was determined with the gensini score
MATERIALS and METHODS:Our patients were consisted of 88 individuals who approached Cardiology Institute of İstanbul University and diagnosed as "stabile angina pectoris"(SAP) by coronary angiography. Biochemical parameters were observed in the biochemistry laboratory of Haseki Education and Research Hospital. Serum PTX-3 was analysed by ELISA kit related with sandwich method.
RESULTS:Group 1 (patients with mild coronary artery diseases and/or gensini score <50)was compared with 2.group 2 (1,2 and 3 vessels affected patients and /or gensini score over 50). The patients who have severe coronary artery disease (Group 2) have distintctly higher ptx-3 levels, found statistically quite significant.
CONCLUSIONS:In our study, it is thought that the statistically high PTX 3 levels are related with atherosclerosis in the evaluation of coronoary artery obstruction degree of SAP patients. Detection of the plasma PTX3 levels of patients diagnosed as SAP before angiography may indicate the severity of the disease thus it may help the detection of atherosclerosis degree and lead to give an angiography decision.
Keywords: Stable Angina Pectoris, Coronary Artery Disease, Pentraxin 3

P-060
Case of Cushing Disease with laboratory findings

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OBJECTIVES:Cushing's disease is a clinical condition of glucocorticoid secretion due to pituitary adenoma secreting ACTH(adrenocorticotropic hormone).The patient, who had been diagnosed with breast cancer before, was diagnosed as Cushing's disease according to the laboratory result during routine biochemistry controls. In this study, we aimed to emphasize the importance of multidisciplinary (laboratory-clinical) approach in the diagnosis of diseases that are directed by biochemical parameters.
MATERIALS and METHODS:A 61-year-old patient with operated breast cancer and diabetes mellitus for 30 years had a fasting blood glucose of 430mg/dl. HbA1c:15.1% Cortisol:28µg/dl(n:5-22µg/dl). 1mg and 2mg dexamethasone suppression test was performed,there was no suppression in the test results. It was not accept by the patient the next step,8mg dexamethasone suppression test. Hospitalization was recommended for further examination and pituitary MR(magnetic resonance) was planned.
RESULTS:MRI findings were interpreted as empty sella or partial empty sella. Inferior petrosal sinus sampling (IPSS) was planned and ACTH levels from the interventional radiology samples were studied.In the sample taken from the left petrosal sinus, ACTH levels were 0.min18.4pg/ml,5.min558pg/ml,10.min778pg/ml; in the sample from the right petrosal sinus 0.min19.8pg/ml,2.min25.8pg/ml,5.min124pg/ml,10.min350pg/ml; in the samples from the peripheral vein 0.min16.4pg/ml,2.min25.3pg/ml,5.min726pg/ml,10.min145pg/ml has been concluded. Based on these laboratory findings, the patient was diagnosed with left lateralized pituitary cushing syndrome.
CONCLUSIONS:In this case, according to the MR findings and the results obtained from IPSS for the diagnosis of Cushing's disease; it was observed that timely and accurate samples are helpful in the diagnosis process and also they save the time for the treatment and follow-up of the patient.
Keywords: Cushing's disease, Inferior petrosal sinus sampling (IPSS)

P-061
Falsely low HbA1c level on the Roche Cobas 6000 platform in a diabetic patient with a high HbF concentration

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OBJECTIVES:Recent episodes of hypoglycemia, Hemolytic anemia or a high percentage of HbF may result in lower than expected HbA1c in diabetic patients.
MATERIALS and METHODS:A diabetic patient (Male, 35) on medication with an average fasting blood glucose of about 157 mg/dl (range 122-195, within two years) recently without any hypoglycemic symptoms had a low normal level of HbA1c (4,6 %, NGSP or 27 mmol/mol, IFCC). Biochemistry and hormone tests were performed on the Roche Cobas 6000 system. To elucidate the discrepancy various hematological and biochemical tests were performed: Complete Blood Count (Sysmex XN 1000) and manual differential count, anemic test panel (Ferritin, Vitamin B12, Folate, Iron/UIBC/Transferrin Saturation), Haemolytic test panel (Coombs tests, LDH, Bilirubins, Reticulocytes) and Hb Electrophoresis (Sebia Capillary Electrophoresis).
RESULTS:The Patient had normal Hemoglobin and red blood cell indices. Due to normal hemolytic test panel results, we ruled out hemolytic anemia. Hb Electrophoresis showed high levels of Hb F 42,1 % (with 57 % HbA and 0,9 % HbA2). According to Roche HbA1c method insert if Hb F is present in more than 10 % than falsely low Hb A1c result may be seen.
CONCLUSIONS:In the absence of recent episodes of hypoglycemia and hemolytic anemia, lower than expected HbA1c values obtained with Roche Hemoglobin A1c may be due to high HbF levels. In these cases, the determination of Hb F percentage by hemoglobin electrophoresis is required. If Hb F is elevated by more than 10% than another method for HbA1c or Glycated Albumin is required for proper glycemia status evaluation.
Keywords: Roche HbA1c, HbF, Haemolytic anemia, Haemoglobin electrophoresis,

P-062
Investigation of homocysteine levels in patients with diabetic nephropathy

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OBJECTIVES:Homocysteine is formed by demethylation of methionine, which is abundant in animal protein and is the core determinant of the methylation cycle. A number of studies also showed that enhanced plasma Hcy level is associated with increasing urinary albumin excretion in diabetic patients. There is also evidence supporting that Hcy abundance is closely related to renal status in the elderly. These results all suggest that Hcy is a marker of impaired renal function in diabetic patients. Our aim of this study is to investigate the levels of homocysteine in patients with diabetic nephropathy and control group.
MATERIALS and METHODS:61 controls and 38 patients with diabetic nephropathy were enrolled to this study. Homocysteine levels were measured by LC-MS/MS. 50 µL plasma, calibrator and control samples were mixed with 50µL in-ternal standard (10µM d8-homocysteine iso-tope DLM-3619-1) and 50 µL reducing reagent (300 mmol/L 1,4-Dithiothreitol) and incubated at room temperature for 15 minutes. 300 µL of precipitating reagent (15% trichloroacetic acid Cat No: Merck 100810) was added to precipitate proteins, mixed for 10 seconds and centrifuged at 13.000 rpm for 3 minutes. 10 µL of superna-tant was injected.
RESULTS:Serum homocysteine levels were significantly higher in patients with diabetic nephropathy (18.7±7.2 µmol/l) than controls ((16.1± 4.8 µmol/l);

p<0.05).
CONCLUSIONS:In our study, we found that serum homocysteine levels were significantly higher in patients with diabetic nephropathy than control group. Therefore, we concluded that homocysteine may be a very useful marker in the diagnosis of diabetic nephropathy.
Keywords: Diabetic nephropathy, homocysteine, LC-MS/MS

P-063
Production and certification of hemoglobin A1c reference material

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OBJECTIVES:Diabetes is a metabolic disorder, which is usually caused by a combination of hereditary and environmental factors and an excessively high level of blood glucose (hyperglycemia). Hemoglobin A1c (HbA1c), also known as glycosylated hemoglobin, is a blood test used to measure the effectiveness of the treatment in diabetes and to diagnose diabetes. Hemoglobin A1c levels are given as percentage (%) in blood by NGSP method. Normally accepted Hemoglobin A1c is between 3 % and 6 %. Hemoglobin A1c levels are measured by various analytical methods. The most commonly used Hemoglobin A1c measurement methods in the literature are 2D-HPLC-CE-UV, HPLC-UV and HPLC-ESI-MS.
MATERIALS and METHODS:Highly precise and accurate liquid chromatography–mass spectrometry (LC–MS/MS) procedure will be developed to measure HbA1c in blood. Also, commutability of HbA1c reference material will be provided by HPLC-UV method as a secondary method for HbA1c measurements.
RESULTS:Firstly, HPLC-UV method was developed for HbA1c measurement in blood. The correlation coefficient (r) of the Calibration Curves obtained was above 0,999 and the accuracy of the Quality Control check was in the acceptance range. We observed no problem at repeatability. Recovery was calculated between 86%-104%.
CONCLUSIONS:HPLC-UV method for measuring HbA1c was developed. Validations of this method has done. After that, to measure HbA1c LC–MS/MS method will be developed. Produced reference material will be certificated with these methods. This certificated HbA1c reference material will be a nationally sourced alternative reference material for clinical area. Also this CRM will reduce outward dependence.
Keywords: Reference material, HbA1c, LC-MS/MS

P-064
Diagnostic distribution of our OGTT results according to American Diabetes Association criteria

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OBJECTIVES:In our study, we aimed to analyze the diagnostic distribution of oral glucose tolerance test (OGTT) results according to American Diabetes Association (ADA) criteria.
MATERIALS and METHODS:Standard oral glucose tolerance test (OGTT) was applied to 298 men and 408 women aged between 18 and 75, who were requested from the outpatient clinics of Kırşehir Training and Research Hospital. Fasting plasma glucose and second hour plasma glucose were measured.
RESULTS:According to the American Diabetes Association (ADA) criteria, 39% of the cases were evaluated as normal (108 males, 172 females), 16% were diabetes (45 males, 70 females), 8% were isolated IFG (impaired fasting glucose) (20 males, 34 female), 33% isolated IGT (114 male, 116 female), 4% IFG + IGT (11 female, 16 male).
CONCLUSIONS:Currently, the incidence of diabetes mellitus and the associated microvascular and macrovascular complications are gradually increasing. 75 g oral glucose tolerance test (OGTT), which is evaluated as proper in accordance with American Diabetes Association (ADA) criteria, has an important role in early diagnosis of diabetes and prevention of complications.
Keywords: Diabetes mellitus, OGTT

P-066
Homocitrulline: Will it be a marker of diabetic nephropathy?

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OBJECTIVES:In the case of carbamylation, isocyanic acid reacts in an irreversible manner with α-and ε-amino groups of proteins, generating α-carbamylated proteins and homocitrulline (HCit,ε-carbamoyllysine) residues, respectively. Our aim of this study is to determine serum homocitrulline levels in patients with diabetic nephropathy.
MATERIALS and METHODS:103 diabetic nephropathy and 35 controls were included. 250 µL of serum and 100µL of D4-L Citruline were vortexed by pipetting with 1000µL of Methanol. The samples were allowed to incubate at room temperature for 10 minutes. It was then centrifuged at 13000rpm for 5 minutes. The supernatant was transferred to a glass tube and the sample was evaporated under 65 degrees nitrogen. 200 µL of 3N HCl + N-Butanol was added to the tube. The cap of the tube was closed and allowed to incubate at 65 degrees for 30 minutes. The sample was evaporated again under nitrogen. 250µL of 20% acetonitrile was dissolved with 0.1% formic acid. Phenomenex Luna C18 column and ABSCIEX API 3200 LC-MS/MS were used for the measurements.
RESULTS:Serum homocitrulline levels were higher in patient group [255 (124-415) ng/mL] compared to controls [248 (103-884) ng/mL] (p=0.009).
CONCLUSIONS:Like glycation process, carbamylation might be responsible for the prognosis of kidney disease in diabetes mellitus. Thus, a carbamylation biomarker, homocitrulline, may be considered as a alternative candidate test.
Keywords: Homocitrulline, Tandem mass spectrometry, Nephropathy

P-067
Evaluation of Th22 and Th9 Cells in Patients withType 1 Diabetes Mellitus

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OBJECTIVES:T helper (Th) cells and their cytokine secretions are thought to have roles in pathogenesis of type-1 DM. Their frequencies are claimed to change in relation with disease progression while they are thought to be contributors of immune attack to pancreatic beta cells.
MATERIALS and METHODS:Heparinised venous blood samples (20 ml) were drawn from patients with type-1 DM (n=20) and healthy controls (n=10). The mean age+Standart Deviation (SD) of the patients with type-1 DM and healthy controls were 29.3±5.6 years (10 males-10 females), 28.4±4.6 years (5 males-5 females), respectively. Duration of disease is 7.5±3.7 years. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-gradient centrifugation from whole blood. PBMCs were incubated with the PMA (50 ng/ml) and Ionomycin (1 mg/ml) for 4 hours at 37 °C with 5% CO_2. Before incubating, Brefeldin A (3 µg/ml) was added as well. After 4 hours, cells were harvested and stained for surface molecule expressions of CD3, CD4. In addition, the intracellular staining was performed for the expression of IL-22 and IL-9. Expression of cell surface and intracellular markers was assessed using flow cytometry whose name is BD FACSCantoII, and data were analyzed by FACSDiva software.
RESULTS:Frequencies of IL-22 and IL-9 of CD3+CD4+ Th cells in patients with type 1 DM were significantly increased compared to healthy controls (p= 0.003 and p=0,022).
CONCLUSIONS:These results show that frequencies of IL-22 and IL-9 cytokines of Th may have roles in pathogenesis of type-1 DM.
Keywords: Diabetes, T helper, IL-22, IL-9

P-068
Correlation between platelet MPV and HbA1c among Bosnian children with type 1 diabetes mellitus

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OBJECTIVES:Diabetes mellitus, regardless of the type, is a prothrombotic state characterized by platelet hyperreactivity and hyperaggregability.Mean Platelet Volume (MPV) is considered a hallmark of impaired thrombopoiesis in diabetes mellitus. Since the data related to diagnostic significance of MPV are lacking and contradictory, in this work, we aimed to compare platelet morphology among children in Bosnia and Herzegovina with Type 1 Diabetes Mellitus (T1DM) and their healthy peers and to analyze possible correlation between platelet morphology and glycated haemoglobin (HbA1C).
MATERIALS and METHODS:The study included 100 children with T1DM and 100 non-diabetic, healthy children as control group. The control group was age- and sex-matched to the study group. In both groups, platelets (x109/L), MPV (fL), HbA1C (%) and glucose (mmol/L) were analysed.
RESULTS:There was no significant difference in BMI and platelet count values between the groups, while HbA1c, glucose and MPV values showed significant differences (p=0.0001 for all three). HbA1c, glucose and MPV were significantly higher in children with T1DM in comparison to healthy children. Positive correlation was observed between MPV and HbA1c (R=0.146, p=0.039), and MPV and glucose (R=0.199, p=0.005).
CONCLUSIONS:MPV is significantly higher in Bosnian children with T1DM when compared to controls. Positive correlation between MPV and HbA1c suggests that MPV levels may serve as a early, inexpensive marker for determining the risk of diabetic microvascular and macrovascular complications.
Keywords: Type 1 diabetes mellitus, Hemoglobin A1c, Mean Platelet Volume, Inflammation marker

P-069
Association between NGAL and glycemic control in patients with type I diabetes mellitus

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OBJECTIVES:Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin protein family. NGAL might be an important factor in the pathophysiology of micro- and macro-vascular complications in patients with poorly controlled diabetes. The aim of the study is to evaluate the levels of plasma and urinary NGAL (pNGAL and uNGAL) as a marker for risk of complications in patients with type 1 diabetes mellitus (T1DM) compared to HbA1c values.
MATERIALS and METHODS:The study included 38 patients with T1DM lasting for more than 5 years, classified into two groups according to the HbA1c: good (< 7.5%) and poor glycemic control (>7.5%). pNGAL and uNGAL were quantitatively measured in plasma and a spot urine sample using particle-enhanced turbidimetric immunoassay (BioPorto).
RESULTS:The group consists of 38 children (22 female and 16 male), the middle age is 14.2±2.5 years. 34.2% of the patients were with good and 65.8% with poor glycemic control. The difference in pNGAL levels between the two groups did not reach a statistical significance. The mean uNGAL/Creatinine ratio (NCR) levels were significantly higher in the group with poor glycemic control (6.06±8.32g/mmol vs 1.65±0.98g/mmol, p=0.001). Pearson correlation analysis showed significant positive correlation between NCR and: HbA1c (r=0.549, p=0.001), Albumine/Creatinine ratio (ACR) (r=0.551, p=0.001), Triglycerides (r=0.395, p=0.025); and negative correlation with HDL-cholesterol (r=-0.355, p=0.046).
CONCLUSIONS:NCR levels are higher in patients with poorly controlled diabetes probably in response to tubulointerstitial renal injury. More studies are needed to clear out the role of NGAL as an early marker for diabetic nephropathy.
Keywords: NGAL, Type I diabetes mellitus

P-070
A biochemistry education survey study with pregraduate medical school students

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OBJECTIVES:The medical biochemistry lectures are processed within the first 3 years of the whole period of our University's faculty of medicine. The survey which includes questions about preanalytic phase, diseases and biochemistry is aimed to be carried out in the group of the pregraduate students.
MATERIALS and METHODS:The study designed as a cross-sectional. Data collection instrument prepared by the researchers themselves. The instrument included 22 items have multiple choice options. The data collected from the students were entered into a standard data base by the researchers. The questionnaire has been directed in to 120 pregraduate student in Izmir Katip Celebi University Medical Faculty. For the analysis of the study data, descriptives statistics were used. Data analysis performed using PASW statistics for Windows (SPSS,Inc.IBM) version 21.0.
RESULTS:%37.5 of the participants don't have the knowledge about correct tube for coagulation and hemogram. The urine collection method of 24 hours and urine sample type for special analysis couldn't be known by the students. %40 of students didin't know the correct sample for prenatal screening.
CONCLUSIONS:In order to correct examination process, to minimize the preanalytic failure range, to evaluate the analysis outcomes more accurate after the graduation period, it will be very effective if the medical biochemistry education is added into the rotation programme before the graduation.
Keywords: medical biochemistry education

P-071
Increased circulating levels of cardiotrophin-1 in women with polycystic ovary syndrome

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OBJECTIVES:Cardiotrophin-1, a member of the interleukin-6 family of cytokines, protects several organs from damage by promoting survival and anti-inflammatory effects. Polycystic ovary syndrome (PCOS) is a reproductive and metabolic disease associated with increased risk of cardiovascular events. The aim of this study was to estimate serum cardiotrophin-1 levels in women with PCOS and to find possible relationships between cardiotrophin-1, insulin resistance and biochemical parameters in these patients.
MATERIALS and METHODS:Forty-six women with PCOS and 36 age matched healthy women were participated in this case-control study. Serum insulin level, homeostasis model assessment of insulin resistance (HOMA-IR), and biochemical parameters were measured. Serum cardiotrophin 1 levels were measured using sandwich-enzyme-linked immunosorbent assay.
RESULTS:Cardiotrophin-1 levels were significantly higher in the PCOS group than in the control group (269 ± 188 pg/ml vs. 177 ± 136 pg/ml, p = 0.01). In addition, HOMA-IR, serum insulin, triglyceride and testosterone levels were significantly higher in the patient group than in the control group. Cardiotrophin-

1 levels in the serum of women with PCOS patients were positively correlated with serum insulin and HOMA-IR.
CONCLUSIONS:The circulating levels of cardiotrophin-1 was significantly increased in women with PCOS. Our results suggest that cardiotrophin-1 has a relationship with insulin resistance in PCOS. Elevated cardiotrophin-1 levels can be a predictor of increased cardiovascular risk in PCOS subjects.
Keywords: polycystic ovary syndrome, cardiotrophin-1, insulin resistance

P-072
Procalcitonin as a biomarker for thyroiditis chronica

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OBJECTIVES:The objective of this case report is to highlight the unusual high level of Procalcitonin (PCT) to help to make the right diagnosis in future cases.
MATERIALS and METHODS:The level of PCT in serum samples was measured using enzyme-linked fluorescence assay (ELFA) B.R.A.H.M.S. PCT Vidas PC Biomerieux. The levels of thyroid hormones,TSH,fT4,fT3,and thyroid antibodies,anti- thyrglobulin and anti-thyroid peroxidase levels in the serum samples were measured using two-site Immunoenzymatic assay Access 2,Beckman Coulter. CRP was measured in the serum samples using turbidimetrc method AU 480 B Coulter. WBC was measured in the whole blood EDTA samples -automated hematology analyzer Sysmex XN 550. Thyroid ultrasound and fine needle aspiration cytology was performed.
RESULTS:After total abdominal hysterectomy 52 years-old women, first day after surgery had a fever (38°C). Levels of PCT in serum sample was 14,1 ng/ml, levels of CRP and WBC were in reference ranges. After antibiotics therapy, measurement of PCT, CRP and WBC were repeated. PCT levels was the 13,9 ng/ml and after 48 hours, 13,1 ng/ml. WBC and CRP were the same. General condition of the patient was good. Levels of TSH, anti-thyroglobulin and anty-thyroid peroxidase in the serum sample were increased (TSH 6,1 µIU/ml,TPO Ab 54,1 µIU/ml,Tg-Ab 17,4 µIU/ml). Levels of fT4 and fT3 were in reference ranges. Thyroid ultrasound detected a thyroid heterogeneous nodule. Fine needle aspiration cytology revealed thyroid follicular benign nodule. Diagnosis was thyroiditis chronica.
CONCLUSIONS:The increased level of PCT may indicate thyroid disease in certain circumstances.
Keywords: Procalcitonin,Thyroid disease,biomarker

P-073
Effect of phenylbutyric acid on obesity induced hypothalamic vasculopathy

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OBJECTIVES:Obesity is a serious metabolic disorder that results from imbalance between energy intake and expenditure. In the central nervous system (CNS), the hypothalamus is a significant brain area in regulating feeding behavior and energy balance. Homeostasis of the CNS microenvironment is maintained by the blood-brain barrier (BBB). BBB is a highly specialized and dynamic barrier. The structural integrity of BBB is sustained mainly by tight junction (TJ) proteins and adherens junctions. Disruption of BBB TJ can lead to impaired BBB function and might initiate vasculopathy. Phenylbutyric acid (PBA) is a chemical chaperone that enhances the capacity of the endoplasmic reticulum and decreases the endoplasmic reticulum stress response signal. It was aimed to evaluate the effect of chemical chaperone PBA on the expression of TJ protein occludin in the hypothalamus.
MATERIALS and METHODS:In the study, lean and ob/ob male mice were divided in two groups (n=8) and administered with either vehicle or PBA for thirty days. After thirty days, all mice were sacrificed and brain tissues were removed. The expression of the TJ protein occludin in the hypothalamus were assessed by western-blotting.
RESULTS:Our initial results demonstrated that the expression of the tight junction protein occludin increased in the hypothalamus of PBA-treated ob/ob mice compared to ob/ob controls.
CONCLUSIONS:The results indicated that obesity induced dysregulation of occludin expression might be compensated via administration of chemical chaperon PBA.
Keywords: Obesity, occludin, phenylbutyric acid, tight junctions, vasculopathy

P-074
The serum levels of TRB3 and sestrin-2 in obese and non-obese patients with polycystic ovary syndrome (PCOS)

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OBJECTIVES:PCOS is an endocrinopathy which is caused to chronic anovulation and anovulatory infertility. Menstruation irregularities, symptoms of androgen excess, obesity and sometimes hirsutism are clinical signs of PCOS. The relationship of between obesity and PCOS isn't explained completely. TRB3 (Tribbles homolog 3) is a mammalian homolog of the drosophila tribble gene. The synthesis of TRB3 increases under various stressful conditions such as endoplasmic reticulum stress and starvation. Increasing of TRB3 causes to hypoglysemia and IR also inhibits adipocyte differentiation. Sestrin-2 is a member of the stress-stimulated protein family which regulates metabolic homeostasis. Sestrin-2 is as a protective antioxidant protein against oxidative stress, ROS and cardiovascular diseases. We predicted that sestrin-2 and TRB levels can be related with metabolic disturbances in PCOS.
MATERIALS and METHODS:57 patients were included who have PCOS to the study. 22 healthy women were enrolled as control group. Patient group was separated to obese and non-obese groups. Metabolic parameters, TRB3 and sestrin-2 tests were performed on patients and control groups. TRB3 and sestrin-2 were measured by microelisa method.
RESULTS:Sestrin-2 mean values were lower in obese PCOS group than non-obese PCOS group ($p<0.005$). In Obese PCOS group, sestrin-2 has negative correlation with HOMA-IR, insülin and BMI. TRB3 mean values were higher in both PCOS groups than control group ($p<0.005$).
CONCLUSIONS:Our study showed that the changes of serum levels of TRB3 and Sestrin-2 is related to metabolic disturbances. These parameters can be used to evaluating of metabolic status in obese and non-obese women with PCOS.
Keywords: Sestrin-2, TRB3, Obesity, PCOS

P-075
Galectin-3 levels and inflammatory response in patients undergoing bariatric surgery

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OBJECTIVES:Obesity is a low-grade systemic inflammatory disease. Galectin-3 is a member of the lectin family and plays a role in inflammatory processes. The aim of our study was to investigate the possible relationship between galectin-3 level and obesity and to evaluate the metabolic inflammatory process before and after obesity surgery through this marker.

MATERIALS and METHODS:Total of 100 patients (normal weight, overweight, 1st, 2nd, and 3rd degree obese) were included in the study. The 3rd degree obese patients were evaluated at 3rd and 6th months after bariatric surgery. In samples taken from all patients, glucose, insulin, HbA1c, lipid profile, high sensitivity C-reactive protein (hsCRP), galectin-3, interleukin (IL) -6, IL-10, adiponectin, and leptin levels were measured.
RESULTS:The average age of the individuals included in the study was 41±9 years. The mean body mass index (BMI) of the 3rd degree obese patients decreased significantly after the 3rd and 6th months of surgery. Galectin-3 levels were higher in the 3rd degree obese individuals compared to the normal weight group. After surgery glucose, insulin, HbA1c and HOMA-IR, IL-6, galectin-3, and hsCRP levels were decreased. IL-6, galectin-3, leptin, and hsCRP levels were found significantly higher in the insulin resistant group (HOMA-IR≥2.5). There was a significant correlation between levels of galectin-3 and IL-6, leptin, and hsCRP.
CONCLUSIONS:In our study, serum galectin-3 levels decreased together with the parameters related to postoperative inflammation and insulin resistance. These findings support that galectin-3 is one of the molecules involved in the linkage between meta-inflammation and insulin resistance.
Keywords: Bariatric surgery, galectin-3, inflammation, insulin resistance, obesity

P-078
Lipid profile in female and male rats subjected to a combined high-fat-high-carbohydrate diet

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OBJECTIVES:High-fat-high-carbohydrate (HFHC) diet is one of the leading etiological factors in obesity, cardiovascular diseases and metabolic syndrome. Animal models are an affordable way to study the negative effects of HFHC diet. They provide a basis for comparison of gender variations. The aim of our study is to compare the effect of the HFHC diet on the lipid profile in female and male rats.
MATERIALS and METHODS:Wistar rats (n = 32) were divided into 4 groups - female and male control (FC and MC) and female and male dietary-manipulated (FD and MD). Groups FD and MD were subjected to a HFHC diet, and FC and MC received standard rat chew, for 16 weeks. At the end of the experiment, after decapitation, mixed blood was collected. Serum concentrations of total cholesterol, HDL- and LDL-cholesterol, and triglycerides were determined.
RESULTS:Compared to the controls, dietary-manipulated groups had higher total cholesterol (1.67 ± 0.1mmol.l-1 vs 2.00 ± 0.1mmol.l-1, P < 0.05), LDL-cholestorol (0.24 ± 0.05mmol.l-1 vs 0.59 ± 0.05mmol.l-1, P < 0.05) and triglycerides (0.99 ± 0.4mmol.l-1 vs 2.48 ± 0.4mmol.l-1, P < 0.05). Compared to females, the male rats had higher total cholesterol (1.68 ± 0.1mmol.l-1 vs 1.98 ± 0.1mmol.l-1, P < 0.05), triglycerides (1.04 ± 0.4mmol.l-1 vs 2.43 ± 0.4mmol.l-1, P < 0.05) and lower HDL-cholesterol (1.36 ± 0.05mmol.l-1 vs 1.06 ± 0.05mmol.l-1, P < 0.05).
CONCLUSIONS:The used HFHC diet increases the serum concentrations of studied lipid parameters in both genders. These disturbances were more pronounced in male rats.
Keywords: lipid profile, hfhc diet, wistar rats, obesity

P-079
Waist circumference as a predictor of atherosclerosis

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OBJECTIVES:Obesity is a complex metabolic disorder which is one of the most common contemporary health problems. Numerous researches show the connection between chronic,low intensity inflammation and obesity, as well as the connection between lipid metabolism disorder and obesity. The aim of this research was to determine connection between waist circumference, lipid status and hsCRP concentration in adult, metabolically healthy subjects.
MATERIALS and METHODS:The research included 82 subjects.in accordance with International association for diabetes mellitus, subjects were divided into 2 groups. The group of subjects with abdominal obesity and control group. Concentration of cholesterol, triglycerides, lipoproteins, hsCRP was measured on the Architect c4000
RESULTS:The average measurements of waist circumference were (100.83±8.12 to 74.68±9.35 cm). Using the Student's test, significantly higher concentrations were observed in group of obese people (cholesterol P<0.001; LDL cholesterol P<0.001; VLDL cholesterol P<0.001; triglycerides P<0.001). By analyzing and comparing the values of HDL cholesterol, significantly lower concentrations of HDL were observed in obese people group. (P<0.001). HsCRP serum concentration was significantly higher in obese subjects (p<0.0001). We established positive correlation between hsCRP concentration and waist circumference, total cholesterol, triglyceride, LDL concentration and waist circumference has been proven, as well as negative correlation between waist circumference and HDL concentration.
CONCLUSIONS:Our results indicate that, given the fact that these changes in lipid profile represent a risk factor in development of atherosclerosis, a proatherogenic lipid profile is favorized in the organism of obese people.
Keywords: Obesity, lipids, atherogenesis, inflamation, hsCRP

P-080
Determination of 8 OHDG levels in metabolic syndrome

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OBJECTIVES:Metabolic syndrome is an important cause of morbidity affecting more and more people both in Turkey and all over the world. Metabolic syndrome is an endocrinopathy in which individuals have multiple factors such as diabetes, impaired fasting glucose, impaired glucose tolerance or insulin resistance.As a result of increasing reactive oxygen species and insufficient antioxidant mechanisms in the body, a number of pathological events called oxidative stress occur. It is known that oxidative stress causes various events and causes damage by showing various effects on DNA by different mechanisms. Therefore, in this study, we aimed to determine the levels of 8 OHDG which are indicative of oxidative DNA damage in individuals with metabolic syndrome and healthy volunteers as control group.
MATERIALS and METHODS:World Health Organization (WHO) diagnostic criteria were used for the diagnosis of metabolic syndrome. In determining 8-OHdG levels, Enzyme-Linked Immuno-Sorbent Assay method was used.
RESULTS:Serum 8-OH-dG (pg/mL) level was found statistically to have increased when compared with those of the control group (0.18±0.14) in METS patients (0.99±0.21).
CONCLUSIONS:In this study, we have tried to show the changes in oxidative stress markers in MetS patients and healthy participants.Many factors that cause metabolic syndrome also trigger oxidative damage. The role of oxidative stress in the pathogenesis of methanolic syndrome needs to be studied and the status of cardiovascular diseases should be demonstrated.
Keywords: Metabolic sendrom, 8-OHdG,oxidatif stress

P-081
Increased asymmetric dimethylarginine levels in patients with Graves' Disease

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OBJECTIVES:Asymmetric Dimethyl Arginine(ADMA) is an endogenous inhibitor of endothelial nitric oxide synthase and reduces nitric oxide release from the endothelium, and causes endothelial dysfunction and local vasospasm. Hashimoto's Thyroiditis is the most common cause of hypothyroidism and is an autoimmune disease caused by antibodies directly attacking the thyroid gland. Graves' disease is an autoimmune disease that causes hyperthyroidism due to hyperactivity of the entire thyroid gland. Non-toxic multinodular goiter(MNG) is an endemic condition in Turkey with multiple nodules in the thyroid gland without increased hormone release.Increased ADMA levels are recently recognized as a novel risk factor for endothelial dysfunction and cardiovascular events.Our aim was to investigate the association between circulating ADMA levels and autoimmune thyroid disorders thought to increase cardiovascular disorders.
MATERIALS and METHODS:A total of 200 euthyroid individuals were enrolled in this prospective study, including 50 patients with Hashimoto's Thyroiditis,50 patients with Graves diseases, 50 individuals with MNG and 50 healthy controls who admitted Selçuk University Medical Faculty between 01.01.2018 and 01.12.2018.Statistical analyses were performed using the IMB SPSS,v21.
RESULTS:ADMA levels were statistically higher in patients with Graves' disease (mean:0.64±0.27 µmol/l) compared to Hashimoto thyroiditis (mean: 0.53±0.18 µmol/l), MNG (mean: 0,51±0,24µmol/L) and control group (mean: 0.49±0.21µmol/l) (p=0.023,p=0.012 and p=0.005,respectively).There were no relationships among ADMA levels, thyroid hormones, TSH or BMI.
CONCLUSIONS:Our study demonstrated that serum ADMA concentrations were significantly increased in patients with Graves' disease, not influenced by gender, age, thyroid hormone levels, BMI and smoking. These findings may explain the biochemical pathway of increased cardiovascular disease in Graves' disease.
Keywords: Hashimoto, Multinodular Goiter, ADMA,Graves' Disease

P-082
Determination of ischemia modified albumin (IMA) level in Hashimoto thyroiditis

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OBJECTIVES:Hashimoto's thyroiditis (HT) is the most common inflammatory disease of the thyroid. The hypothyroid in which Hashimato cases are seen is characterized by a decrease in oxidative metabolism and a significant increase in lipid and lipoprotein plasma levels. This situation causes the balance of metabolism in the organism to be disrupted and oxidant-antioxidant balance changes.Ischemia-modified albumin (IMA) is a biomarker that is an indicator of ischemia and oxidative stress and is measured by albumin cobalt binding test. Several changes that occur at the amino terminal end of human serum albumin during ischemia are caused by oxidative free radicals and in particular reduce the binding capacity of transition metals such as cobalt. Therefore, in this study, we aimed to investigate whether ischemia marker IMA has changed in hashimoto, an autoimmune thyroid dysfunction, and how it affects thyroid damage.
MATERIALS and METHODS:24 patients diagnosed with HT and 25 healthy women were joined in our study. IMA levels were determined by albumin cobalt binding test, a colorimetric method
RESULTS:Plasma IMA level was higher in HT patients compared to controls (0.64±0.11 AU and 0.53±0.14 AU respectively). There was no statistically significant difference between the groups in terms of IMA levels. (p>0.05,p= 0.392)
CONCLUSIONS:When the functions of the thyroid are impaired (both in hypothyroidism and hyperthyroidism), the organism's use of oxygen and thus metabolic events that are primarily responsible for heart ischemia change. Therefore, we think that further research is needed for IMA which is evaluated as an important indicator and evaluated with heart ischemia
Keywords: Hashimoto Thyroiditis, IMA, Oxidative stress

P-083
Diagnostic significance of inflammatory parameters in obese prepubertal and pubertal Bosnian children

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OBJECTIVES:Obesity in pre-pubertal and pubertal children is a serious problem,being connected with systemic low-grade inflammation and endothelial dysfunction and different disorders like metabolic syndrom, insulin resistance, hypothyroidism. The data related to inflammatory markers in these characteristic populations are lacking in Bosnian children, therefore, major aim of this work was to determine differences in concentration of selected inflammatory markers in Bosnian obese pre-pubertal and pubertal children and to define their possible relationship with inflammation.
MATERIALS and METHODS:Body Mass Index (BMI - kg/m2), number of leukocytes (n x109//)L), neutrophils granulocytes, lymphocytes, platelets, as well as neutrophils/lymphocyte ratio, platelet/ lymphocyte ratio, systemic immune-inflammatory index (SII) and C-reactive protein (CRP- mg/L) were analyzed in 115 obese and 100 non-obese children as a control group who were further subdivided into prepubertal and pubertal children.
RESULTS:Significantly elevated BMI, leukocytes, neutrophils/lymphocyte ratio, platelet/ lymphocyte ratio, SII and C-reactive protein (p<0.001 for all parameters) were observed in the group of obese children in comparison to controls. Neutrophil granulocytes, Lymphocytes Neutrophil/lymphocyte (p<0.001), Platelets/Lymphocytes (p=0.016), and SII (p<0.001) were significantly affected by age while leukocytes and CRP were not altered significantly. In the obese group, positive correlation was observed between BMI and: neutrophil granulocytes (r=0.416; p<0.001); SII (r=0.316; p<0.001); neutrophils/lymphocyte ratio (r=0.333; p<0.001) and Platelets/Lymphocytes ratio (r=0.269; p<0.001).
CONCLUSIONS:There is a positive association between BMI and several inflammatory parameters such as neutrophil granulocytes, SII, neutrophil/ lymphocytes and platelets/lymphocytes ratios. Early identification of those biomarkers in defined populations may help in the prevention of obesity associated complications.
Keywords: Childhood obesity, inflammation, inflammatory markers, age

P-085
Serum sclerostin levels in obese children and adolescents

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OBJECTIVES: The basic interactions between obesity and bone is complex and not well known. Research findings suggest that obesity is detrimental to bone health despite potential positive effects of mechanical loading conferred by increased body mass on bones. Recently, the wnt/ beta-catenin signaling pathway

and its one of the inhibitor sclerostin were found to be involved in the control of bone mass. The aim of this study was to investigate the serum sclerostin levels in obese and non-obese children and adolescents and compare with other bone turnover markers and bone mineral density (BMD).
MATERIALS and METHODS: The study included 38 obese children and adolescents (19 males and 19 females) aged from 7 to 17 years and 38 healthy normal-weight controls (18 males and 20 females) aged from 6 to 17 years. Serum sclerostin levels were measured by ELISA method using commercially available kit.
RESULTS: Body mass index ($p=0.000$) and sclerostin ($p<0.05$) levels of the obese children was significantly higher than that of non-obese children.
CONCLUSIONS: Our result of higher serum sclerostin levels of the obese children and adolescent showed a tendency toward bone loss in obese children and adolescents.
Keywords: Obesity, Osteoporosis, Sclerostin

P-086
Vitamin D and lipid profile levels in obesity

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OBJECTIVES:In this study, we aimed to investigate the relationship between vitamin D levels and lipid profiles of obese patients.
MATERIALS and METHODS:The study included 142 people who applied to endocrine polyclinic for weight loss. Patients were divided into two groups according to body mass index. Demographic and laboratory data were obtained from patient files.
RESULTS:The mean age of the 142 participants was 34 ± 6 years. The number of women was 120 (85%), while the number of men was 22 (15%). When obese subjects were compared to non-obese subjects, waist circumference, fat mass, lean body mass, total body water and basal metabolic rate were increased, while high density lipoprotein levels were significantly lower. When fasting blood glucose, HbA1C and insulin resistance were compared between obese and non-obese, there was a significant difference between the two groups. There was no relationship between obesity and gender (Pearson Chi square test 0.435, p = 0.500). There was no significant difference between obese and non-obese groups in terms of vitamin D levels (Mann-Whitney U test 2881, p = 0.663). However, when the groups were divided into three groups as 30 ng / mL according to 25-OH vitamin D levels, there was a statistically significant relationship between vitamin D and obesity (Pearson Chi square test 5.575, p = 0.0179). Serum total cholesterol, TG and LDL levels were lower and HDL levels were higher than patients.
CONCLUSIONS:This may be explained by vitamin D deficiency itself or by differences in vitamin D metabolism during the development of obesity.
Keywords: Vitamin D, Lipid profile, Obesity.

P-088
Insulin Resistance Markers in Polycystic Ovarian Syndrome

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OBJECTIVES:Polycystic ovary syndrome (PCOS) is the most frequent endocrin disorder in reproductive-age woman (5-10%). As a multisystemic, reproductive-endocrinologic disorder that carries long term health risks such as type 2 diabetes, dislipidemia, cardiovascular diseases and endometrial carcinoma, PCOS is a public health issue. Insulin resistance may play important roles in the pathophysiology of PCOS.
MATERIALS and METHODS:Our study group included 53 patients diagnosed with PCOS and 42 healty volunteers. Patient group and control group has been divided into two groups; "normal weight" as BMI < 25 and "over weight" as BMI > 25. Demographic properties of Patient group and control group determined. Hirsutism scoring, pelvic or vaginal US examination performed. Serum glucose, insulin, total testosteron, SHBG, LH, FSH, total cholesterol, triglicerides, HDL cholesterol and LDL cholesterol levels are determined in all individuals. HOMA-IR calculated to determine insulin resistance. SAI calculated for biochemical hyperandrogenism. Adiponectin, ghrelin, resistin and visfatin level determined. Main groups and sub-groups compared.
RESULTS:There was significant difference between control group and patient group in adiponectin, ghrelin, resistin and visfatin levels. Adiponectin and visfatin levels were lower, ghrelin, resistin, LH levels and LH/FSH ratio were higher in PCOS group. Insulin and HOMA-IR was also high. There was significant difference between groups in total testosteron levels and SAI. There was a negative weak correlation between adiponectin and ghrelin.
CONCLUSIONS:Adiponectin, ghrelin, resistin and visfatin may play roles in insulin resistance. In this study, alteration of parameters showing insulin resistance demonsrated that insulin resistance plays an important role in the pathogenesis of PCOS.
Keywords: Polycystic ovary syndrome,adiponectin, ghrelin, resistin, visfatin

P-091
Rethinking common solvents in butyrylcholinesterase activity assays

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OBJECTIVES:Butyrylcholinesterase (BChE) plays a secondary or supportive role in cholinergic neurotransmission and is recognized as a therapeutic target in the fight against Alzheimer's disease. Today, there is a growing interest in identifying natural or synthetic small-molecule BChE inhibitors, whose drug-likeness is investigated both by binding and enzyme kinetic studies. In BChE activity assays, these potential drug candidates are normally dissolved in one of several water-miscible organic solvents. However, the inhibitory effects of common solvents on BChE remain largely unknown. Here, we aim at exploring the inhibitory activities of acetone, acetonitrile, dimethyl sulfoxide, ethanol and methanol against mammalian BChE.
MATERIALS and METHODS:BChE activity was assayed colorimetrically using butyrylthiocholine (BTCh) as substrate and dithiobisnitrobenzoate as chromogen. The kinetic parameters and mode of inhibition were determined statistically by nonlinear regression (curve-fitting), and the data were then graphed in Lineweaver–Burk and Dixon plots for illustration purposes.
RESULTS:Our results show that all of the solvents tested inhibit BChE in a dose-dependent manner, albeit to varying extents. Methanol is the least potent inhibitor of the enzyme ($IC50$ = 12,199 mM, or ~49% (v/v)) at 1 mM BTCh, while acetone is the most potent inhibitor of the enzyme ($IC50$ = 707 mM, or ~5% (v/v)) at 1 mM BTCh. The mode of BChE inhibition by acetone is best described as competitive with respect to BTCh.
CONCLUSIONS:Our findings suggest that great care must be taken in BChE activity assays using acetone in particular to ensure that solvent-related inhibitory effects do not conceal the true kinetics of BChE–inhibitor interactions.
Keywords: butyrylcholinesterase, Alzheimer's disease, enzyme inhibition, solvent-related effects, acetone

P-092
The relationship of autoantibody aganist erythrocyte antigens with macroenzymes

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OBJECTIVES: In this study, the relationship between auto antibodies against erythrocyte antigens and these macromolecules will be investigated.
MATERIALS and METHODS: The study included 35 patients with auto antibody positive using gel Centrifugation and colon agglutination methods and 35 healthy donors who came for blood donation. ALP, AMY, AST, CK, GGT and LDH tests were measured before and after precipitation with 25% polyethylene glycol using serum from all participants. Recovery calculations were made after precipitation with PEG.
RESULTS: It was found that the recovery of AMY and LDH was lower in patients with auto antibody positive compared to the control group (respectively, AMY: 0.64±0.09, 0.49±0.18 and LDH: 0.64±0.09, 0.49±0.18; $p<0.001$

CONCLUSIONS: The presence of auto antibodies against erythrocyte antigens is associated with the formation of macromolecules, which are believed not to show complete biological activity, particularly in AMY and LDH.
Keywords: Macro lactate dehydrogenase, Macro amylase and Auto antibody

P-093
Detection of thiopurine S-methyltransferase mutations by Multiplex PCR

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OBJECTIVES: Thiopurine methyltransferase gene is located on chromosome 6 and is approximately 34 kb in length. More than twenty eight genetic variants have so far been identified, the majority of which are associated with low levels of TPMT activity. The aim of this study was set up a PCR based method for screening of TPMT mutations.
MATERIALS and METHODS:Whole-blood samples collected into 4 mL EDTA tubes. Genomic DNA was isolated from each blood samples using AccuPrep Genomic DNA Extraction Kit (Bioneer). Two reactions containing a mixture of wild-type primers, mutation-specific primers and a pair of positive control primers were performed on genomic DNA. Multiplex PCR PreMix tubes (Bioneer) consisted of a total volume of 20 µL containing 200 µmol/l dNTP, 0.5 µM of each primer, 4.0 mmol/l $MgCl_2$, 1 U of Taq DNA polymerase, and 100 ng of genomic DNA. Temperature cycles (30 in total) were 94°C for 30 s, 65 °C for 30 s, and 72 °C for 40 s. PCR products were run by 1.5% agarose jel electrophoresis. Amplified DNA fragments were visualised using ethidium bromide, under UV light.
RESULTS:We set up multiplex, allele-specific polymerase chain reaction (PCR) method that detects the 238G>C, 460G>A, and 719A>G mutations, allowing for identification of TPMT*2 and TPMT*3 alleles.
CONCLUSIONS:Molecular diagnosis of TPMT polymorphism is a strong alternative for enzyme activity assays. This multiplex PCR assay for common TPMT mutations is simple, rapid, accurate, and cost-effective option for screening of patients in clinical research studies.
This project was supported by Çukurova University Research Projects Unit(FDK-2019-11950).
Keywords: Thiopurine methyltransferase, 6-thioguanine, 6-methylthioguanine

P-094
Inhibition of alkaline phosphatase on glyphosate and the effect of some molecules on this inhibition

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OBJECTIVES:Alkaline phosphatase is a zinc metalloenzyme which hydrolyze organic phosphate esters from phosphate group in alkali medium. Although glyphosate as a herbicide, which is thought to cause cancer, kidney damage and many neurological damages, is allowed to be used in the European Union for another 5 years, it is first completely banned in Austria. Since glyphosate has a phosphate structure, we aimed to carry out activity studies considering that it would have inhibitory effect on alkaline phosphatase.
MATERIALS and METHODS:Alkaline phosphatase activity was modified by Bower and McComb's method and endpoint measurement was performed. Two different buffers with glycine and 2A2M1P were used for determination of alkaline phosphatase in human serum. The inhibitory effect of glyphosate(282mg/L) on alkaline phosphatase, as well as the effect of dexamethasone(160mg/L), alendronate(12.8mg/L) and deoxycholic acid(654mg/L) on inhibition with glyphosate were investigated.
RESULTS:Glyphosate activity in glycine buffer(83%) was decreased more than 2A2M1P(%94). It has been observed that deoxycholic acid decrease alkaline phosphatase activity in both buffers and potentiates the inhibition effect of glyphosate. Dexamethasone was measured to reduce the inhibitory effect of glyphosate in glycine buffer. Alendronate did not alter the inhibitory effect of glyphosate in the glycine buffer but caused an increase in the slight inhibitory effect of glyphosate in the buffer with 2A2M1P(90%).
CONCLUSIONS:Alkaline phosphatase activity differed between the two buffers. The presence of glycine in the glyphosate structure may have reduced the inhibitory effect on activity in the glycine buffer. In addition, dexamethasone decreased glyphosate inhibition and alendronate wasn't effective in inhibition, suggesting further studies.
Keywords: Glyphosate, Alkaline Phosphatase, Enzyme Inhibition

P-096
Investigation of homocitrulline levels in healthy people and patients with Behçet's Disease

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OBJECTIVES:Behçet's disease (BD) is a multisystemic and inflammatory disease characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis, epididymitis, mucocutaneous, joint, gastrointestinal, neurological, and vascular involvement. In this study; we aimed to investigate the levels of serum homocitrulline, a characteristic carbamylation-derived product, in BD and healthy people.
MATERIALS and METHODS:This study was performed with 30 control subjects and 30 patients with Behçet's disease, who admitted to the Selcuk University Faculty of Medicine, Department of Rheumatology. Serum homocitrulline and lysine levels were determined by liquid-chromatography tandem mass spectrometry. Statistical analysis was performed with SPSS v16.
RESULTS:The homocitrulline levels of the patient group (1.11±0.74 mmol/mL) were significantly higher (p=0.001) than control group (0.38±0.12 mmol/mL). Homocitrulline/lysine ratios were higher in the patient group (1.15 ± 0.89 mmol/mol) compared to the control group (0.31 ± 0.10 mmol/mol) (p<0.001). However, lysine analysis showed no significant difference between groups.
CONCLUSIONS:In this study that was performed for the first time, there was a positive relationship between behçet disease and homocitrulline levels. Therefore, it is thought that homocitrulline levels may be used as biomarkers in behcet disease.
Keywords: Behçet's disease; Homocitrulline; Inflammation

P-097
The relationship between ischemic-modified albumin level in patients with Ankylosing Spondylitis

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OBJECTIVES: Ankylosing spondylitis (AS) is a chronic inflammatory disease of the spine and sacroiliac joint with unknown etiology. Inflammation is associated with increased oxidative stress; recent studies have implicated increased oxidative stress in the pathogenesis of AS. Ischemic-modified albumin (IMA) is an altered form of albumin and increases in oxidative stress. The aim of this study was to investigate the IMA levels and the relationship between in AS.
MATERIALS and METHODS:The study included 63 patients (28 female, 35 male) diagnosed with AS according to Modified New York Criteria and 48 participants (22 female, 26 male) as healthy controls. The patients and controls had no known cardiovascular risk factors. Both groups were examined for serum protein, albumin, lipid profile, C-reactive protein (CRP), and hemogram. Serum IMA levels of the groups were compared.
RESULTS:The patient and control groups were similar in terms of age and gender. Serum IMA levels were significantly higher in the patient group than in the controls. Among the patients with AS, serum IMA levels were significantly

higher in those with active disease (BASDAI ≥4). The IMA and CRP levels were positively correlated in the patients with active disease.
CONCLUSIONS:Higher levels of IMA in patients with AS or in those with active disease suggest that it may be associated with pathogenesis and activity of the disease. However, more comprehensive studies with larger number of patients would be necessary in order to evaluate the IMA level as an inflammatory marker in AS.
Keywords: ischemic-modified albumin, ankylosing spondylitis, c-reactive protein

P-098
Short-term effects of sleeve gastrectomy on metabolic variables in obese patients

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OBJECTIVES:The objective of this study was to determine the short-term effects of sleeve gastrectomy on some metabolic health variables in the blood of obese patients.
MATERIALS and METHODS:A total of 9 patients (men, 3; women, 6) with obesity (BMI ≥30 kg/m2) visiting Pamukkale University Hospital from March to July 2019 were included in this study. Blood samples were collected before and after 2 months of sleeve gastrectomy. Levels of serum hepatic enzymes, and serum sodium, potassium, and chloride levels were determined by spectrophotometric procedures. The whole blood and sera were analyzed for Glycated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TGs), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Urea, creatinine and blood urea nitrogen levels were also detected.
RESULTS:Serum urea and creatinine contents were significantly ($p < 0.05$) decreased in postoperative obese patients compared to preoperation. Remarkable improvements in perturbed metabolic variables approaching normality were perceivable. Serum albumin and total bilirubin concentrations were significantly increased ($p < 0.05$).
CONCLUSIONS:Obesity, defined as a multi-factor disease which is very common in all over the world. Obesity resulted in perturbations of whole body metabolism. Sleeve Gastrectomy is a widely applied surgical procedure which aimed the weight loss in obese people by reducing the stomach volume. Metabolic parameters were normalized and improvements in the general health status of the patients were observed in a short-term period after sleeve gastrectomy.
Keywords: Obesity, Sleeve gastrectomy, Metabolic variables

P-099
Correlation between serum levels of Anti CCP and RF in patients with joint pain

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OBJECTIVES:Rheumatoid arthritis affects 0.3% to 1% of the population. It is an autoimmune disease characterised by chronic synovial inflammation. Currently, the most well-known and established test is Anti CCP. It is extremely specific for rheumatoid arthritis, and is present early in disease, and predicts the erosive states of disease.
MATERIALS and METHODS:This prospective interventional study was performed between January 2019 and May 2019 in the PHO Clinical Hospital in Bitola. The study included 30 subjects - 21 females are with join pain and 9 males. The blood samples were taken after overnight fast (12 hours). Anti CCP and RF were determined by Abbot Architect CI 4100 analyzer.
RESULTS:We found increased level of Anti CCP in 6 patients (4 females, 2 males), 11 patients have increased level of RF (7 females, 4 males), 3 patients have increased level of Anti CCP and RF (2 males, 1 women) and 16 patients have normal values of Anti CCP and RF. We found a great correlation in 19 patients between Anti CCP and RF
CONCLUSIONS:We found a significant correlation between RF and Anti CCP.
Keywords: anti-cyclic citrullinated peptides (anti-CCPs), rheumatoid factor, rheumatoid arthritis

P-100
The role of netrin-1 in rheumatoid arthritis

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OBJECTIVES:Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that primarily affects the lining of the synovial joints and is associated with progressive disability, premature death, and socioeconomic burdens. Netrin 1 was initially identified as an axon guidance factor, and recent studies indicate that it inhibits chemokine-directed monocyte migration. Despite its importance as a neuroimmune guidance cue, the role of netrin 1 in osteoclasts is largely unknown. Recent studies have shown high levels of netrin 1 in rheumatoid arthritis patients. The aim of this study is to clarify the role of Netrin-1 in the diagnosis, progression of RA.
MATERIALS and METHODS:34 control and 45 patients with RA were enrolled to this study. Collected serum samples were stored at −80°C, then analyzed for netrin-1 by ELISA (kit from USCN Life Sciences Inc.).
RESULTS:Serum Netrine-1 levels were significantly higher in patients with RA (2775.14(437.25-6226.16)) than controls (589.14(235.13-869); $p<0.001$).
CONCLUSIONS:We concluded that netrin-1 can be a useful marker in the diagnosis of rheumatoid arthritis. However, further studies with larger clinical groups are necessary to identify the possible relation between netrin-1 and pathogenesis of RA.
Keywords: Netrin-1, rheumatoid arthritis, inflammation

P-101
Investigation of netrin 1 levels in Behcet's Disease

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OBJECTIVES: Behçet's Disease (BD) is a rare systemic vasculitis disorder of unknown etiology characterized by recurrent attacks of oral aphthous ulcers, genital sores, and ocular lesions (triple-symptom complex). Recurrent attacks of acute inflammation characterize Behçet's disease. Netrin-1, a secreted laminin-like protein identified as an axon guidance molecule. Netrin-1 regulates inflammation but the mechanism by which this occurs is unknown. Our aim of this study is to investigate the role of Netrin-1 in the diagnosis of Behcet's disease.
MATERIALS and METHODS: The study was conducted with 35 controls and 35 patients with Behçet's disease. Serum samples were stored at -80 ° C until analysis, serum netrin-1 levels were analyzed by ELISA (kit from USCN Life Sciences Inc.).
RESULTS: Serum Netrin-1 levels were significantly higher in patients with Behcet's disease (3732 ± 934.99) than control group (524.88 ± 160.83); $p<0.001$).
CONCLUSIONS: In our study, serum Netrin 1 levels were significantly higher in patients with Behçet's disease than control group. Therefore, we concluded that netrin 1 may be a useful marker in the diagnosis of Behcet's disease.
Keywords: Netrin-1, Behcet's disease, inflammation

P-102
Level of serum adiponectin in Sjögren's Syndrome

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OBJECTIVES: To evaluate the serum adiponectin level and determine the association between adiponectin and various clinical and laboratory findings in patients with primary Sjögren's syndrome (pSS).
MATERIALS and METHODS: A total of 50 patients and 30 healthy volunteers were enrolled in the present study. Serum adiponectin levels were detected by colorimetric enzyme-linked immunosorbent assay. The medical history of patients including complete blood count analysis; high sensitive C-reactive protein; erythrocyte sedimentation rate (ESR); complement component 3; complement component 4; low density lipoprotein cholesterol; triglyceride; immunoglobulin G (IgG), IgA, and IgM levels; and the status of Ro 60, Ro 52, Sjögren's syndrome A, Sjögren's syndrome B, and rheumatoid factor were obtained from laboratory information system.
RESULTS: Serum adiponectin levels were 2.34 (0.77–4.95) ng/mL and 1.73 (0.01–7.76) ng/mL in patients and controls, respectively (p=0.316). Positive correlation was observed between the values of serum adiponectin, ESR (p=0.013, rho=0.362), and body mass index (p=0.018, rho=0.362) in patients.
CONCLUSIONS: These findings indicate that adiponectin does not play a crucial role in the immunological and clinical patterns of pSS.
Keywords: Sjögren's syndrome, adiponectin

P-103
Serum resolvin E1 levels and its relationship with disease activity in ulcerative colitis

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OBJECTIVES:Resolvins originate from ω-3 PUFA (polyunsaturated fatty acid) precursors and play a role in the resolution of inflammation. The aim of this study was to determine the serum ResolvinE1 levels in patients with ulcerative colitis (UC) and to evaluate the relationship between the serum ResolvinE1 levels and ulcerative colitis disease activity.
MATERIALS and METHODS:Serum samples were collected from 51 patients with UC and 30 healthy controls for the determination of Resolvin E1 levels. Firstly, we compared the serum Resolvin E1 levels between the UC patients and the control group. Subsequently, Resolvin E1 levels were analyzed in patients with active UC and UC in remission. Finally, the correlation between Resolvin E1 and C-reactive protein (CRP) and partial Mayo score (p-MS) was analyzed to determine the efficacy of Resolvin E1 in predicting disease activity
RESULTS:Serum Resolvin E1 level was determined in the UC group (3126 ± 1413 ng/ml) and in the control group (2758 ± 1065 ng/ml) (p = 0 187). Serum Resolvin E1 levels were determined in patients with active UC (3114 ± 1166 ng/ml) and patients in remission (3132 ± 1520 ng/ml) (p = 0 749). In the UC group, a low-grade positive significant association was found between Resolvin E1 and CRP (r = 0 303, p = 0 031). There was no significant association between Resolvin E1 and partial Mayo score (r = −0 207, p = 0 146).
CONCLUSIONS:There was no sufficient evidence that Resolvin E1 was an appropriate inflammatory marker to determine disease activity in UC.
Keywords: resolvin e1, inflammotory bowel disease, ulcerative colitis, inflammation, biomarke

P-104
The role of inflammation on vascular endothelial growth factor in patients on peritoneal dialysis

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OBJECTIVES:: Synthesis of vascular endothelial growth factor (VEGF) is under the influence of a chronic peritoneal dialysis process due to which VEGF is found in the drained dialysate (dd). The objective of this prospective study was to evaluate the concentration of vascular endothelial growth factor (VEGF) in serum and ddVEGF during the first six months of the PD, as well as the relationship between these concentrations and demographic and biochemical parameters, the presence of diabetes, peritonitis and the use of drugs.
MATERIALS and METHODS:The study included 20 patients, with an average age of 62.9 ± 12.69, of whom 11 were ill with diabetes. Blood samples were taken at the beginning and after six months of PD, in a vacutainer without additives.
RESULTS:After six months of PD, concentrations of sVEGF increased significantly without significant change in ddVEGF. Concentrations of sEGEGF at the onset of chronic PD treatment directly correlated significantly with serum fibrinogen, and after six months with fibrinogen and glycemia. Patients who received angiotensin-converting enzyme (ACEi) inhibitors had sVEGF and ddVEGF levels slightly below those who did not use ACEi, however sVEGF increased significantly over six months PD. After six months of PD, ddVEGF was significantly higher compared to those who did not use ACEi. Treatment with statins did not significantly affect the levels of sVEGF and ddVEGF during monitoring.
CONCLUSIONS:VEGF serum concentrations and drained dialysis in PD patients are associated with a weaker metabolic profile, while the role of inflammation and treatment agents needs to be further studied.
Keywords: VEGF, Peritoneal dialysis

P-105
Red blood cell distribution width as a biomarker of inflammation

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OBJECTIVES:Recent studies have demonstrated that red cell distribution width (RDW) is associated with inflammation and it can serve as a potential parameter for inflammation. The aim of the study was to investigate the relationship between RDW levels and some traditional inflammation biomarkers.
MATERIALS and METHODS:We retrospectively retrieved 8354 patients' RDW, erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP) and serum ferritin results for six-month period from the laboratory information system. Patients were divided into two groups in terms of their RDW values. Patients with RDW results <14.5% were determined as the first group (n =3559) and those with ≥14.5% as the second group (n=4795).
RESULTS:CRP and ESR levels in the second group were found to be statistically significant higher than the first group (p<0.001). Ferritin levels were higher in the first group but there was no significant difference between the two groups (p=0.059). There were a positive, but weak correlations between RDW and CRP (p<0.001, r=0,215); RDW and ESR (p<0.001, r=0,158).
CONCLUSIONS:Our study showed a possible relation of RDW with CRP and ESR. RDW may be a useful diagnostic marker of inflammation and should be confirmed with follow-up studies in future.
Keywords: Inflammation, red blood cell distribution width (RDW), CRP, ESR.

P-106
Caspase 3, 8, 9 and granzyme B activities in asthma patients

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OBJECTIVES: Asthma is a heterogeneous disease characterized by chronic airway inflammation associated with airway hypersensitivity to direct or indirect stimuli. There is strong evidence that apoptosis dysfunction may play an important role in the pathogenesis of asthma-induced airway inflammation. Therefore, it is important to understand the pathways of apoptosis and the role of apoptosis in the pathogenesis of asthma. This study aimed to determine how apoptosis biomarkers in asthma patients are affected, and also in the diagnosis of the disease whether apoptosis biomarkers may be used as blood-based biomarkers.
MATERIALS and METHODS: The patient group (n = 40) consisted of people who were diagnosed with asthma and had not started taking medication. The control group consisted of volunteers who were similar in terms of age and sex to the patient group (n = 40). Serum levels of Caspase-3, caspase-8, caspase-9 and Granzyme B were measured by ELISA method on blood samples collected from patient and control groups.
RESULTS: It was observed that Caspase-3, caspase-8, caspase-9, and Granzyme B levels were higher in the patients' group compared with the control group ($p<0,001$).
CONCLUSIONS: This study demonstrates that increased levels of apoptosis may play a role in the pathophysiology of asthma and that the increase in serum caspase-3, caspase-8, caspase-9, and granzyme B levels may be blood-based biomarkers for apoptosis. However, further studies are needed to understand the role of apoptosis in asthma.
Keywords: Asthma, Apoptosis, Caspase, Granzyme B

P-108
Evaluation of sample quality for coagulation analysis on the Sysmex CS-5100: HIL index

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OBJECTIVES:Hemolysis, icterus and lipemia (HIL) in specimen may affect the reliability of coagulation test results. This possible interference can be influenced by several factors including the level of interfering substance in plasma, the assay principle and the end-point detection system, that is optical versus mechanical detection. The aim of this study was to determine frequency of HIL in patients' sample for coagulation assays.
MATERIALS and METHODS:We assessed 7712 patients' sample over a two month period and determined the incidence of HIL, reling on the manufacturer to document HIL estimates on instrument. Plasma samples were run on CS5100 autoanalyser (Sysmex, Japan), photo-optical clot detection. The instrument identifies HIL specimens with a specific flag. The quality of the sample is automatically detected with a combination of the multi-wavelength detection method and HIL detector.
RESULTS:Percent of hemolyzed specimens was determined as 1.18%. We also identified the frequency of lipemia as 0.3%. Total of 533 samples (6.9%) were icteric. Due to severe lipemia and hemolysis, 7 and 8 of samples were rejected, respectively.
CONCLUSIONS:The laboratories must monitor and evaluate the quality of the samples and identified problems. Quality results are dependent on quality of specimen. Visual evaluation of the sample is not appropriate because there is significant inaccuracy and inter-individual variation in this type of assessment. HIL may interfere the optical instuments. These interferences can be determined by coagulation analyzer, possessed HIL detection system using multiwavelength light and incorrect results prevented. A test-based interference approach may be useful to avoid unnecessary sample repetition.
Keywords: Coagulation, hemolysis, icterus, lipemia

P-111
Cost analysis and capacity assessment of medical laboratory in Ankara between 2013-2017

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OBJECTIVES:We aimed to evaluate the laboratory income and expense analysis in Ankara province of the Public Hospitals Association based on employee resource, population density and foreign currency.
MATERIALS and METHODS:Laboratuvary service procurement, procurement of goods and total expenses and income were obtained from TDMS between2013-2017. The number of laboratory tests, number of working physicians and technicians, total number of outpatients and inpatients, population density and growth rates were also evaluated. The ratio of laboratory costs within total health expenditures and the change in years was also calculated.
RESULTS:The number of tests between the years were 67.897.658-72.922.524-74.610.415- 82.749.391 and 112.261.365, respectively. Expense per test increased from1.35T.L. to 1.50T.L. Service procurement and material purchase rates in laboratory expenses were 60%-40% in 2013, this ratio completely reversed in 2017.Between these years, the ratio of laboratory expenses in total health expenditures was 5,46%-5,12%-4,84%-5,41% and 5,89%,respectively. While the population density increased 8%, the number of tests increased 65% and the number of polyclinics increased 55%. The number of tests per person and the number of policlinics per person were 13,45-14,15-14,15-15,47-20,61 and 3,35-3,56-4,10-4,41-4,81, respectively. The number of physicians increased from 374 to 512 while the number of technicians increased from 950 to 976.
CONCLUSIONS:The fact that the increase in the number of policlinics and the number of tests is not parallel to the population growth rate,which reflects the increase in health service application and the tendency to seek further investigations. All these findings will shed light on the determination of future health policies.
Keywords: Cost analysis, Procuerment, Laboratory, Health Service, Population Density

P-112
Setting the relevant quality indicators from pre-analytical phase in an emergency clinical hospital

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OBJECTIVES:The study aim was to identify the relevants quality indicators-QIs to inhance patient safety by continous improvement of our clinical laboratory activity.
MATERIALS and METHODS:Prospective study, carried out for 18 months for in-patients, by analyzing collected data on e-requests and types of biological samples received for clinical chemistry and hematology compartments.The calculated values for the 12 selected Qis were expressed as %, defects per million -DPM and on six sigma scale.
RESULTS:During the follow-up time we had received 29454 request forms and 36746 biological samples. The data analysis of selected Qis values showed the highest % rate for the Pre InpMT and the PreMisR (0.89% and 0.94%), respectively the lower one for the PreUnIns (0.065%).The 3.9 Sigma score value associated to the the critical errors corresponding to the Pre InpMT and to PreMisR showed an immediate need to staff training as mandatory corrective action.We have obtained a 4.4 sigma score for the hemolysed primary

samples. Reporting errors Sigma score 4.4 associated to the biological samples for hematology compartment was over the 4.2 value obtained for the clinical biochemistry specimens. We proved a good performance by the Sigma score between 4.1 to 4.4 for 8 monitored Qis, but the accuracy improvement of entering data process in e-request form it's a must.
CONCLUSIONS:Study results were used as entry data for management analysis to ensure risk mitigation especially in the extraanalytical phase by improving communication and training of clinical staff in order to increase lab performance
Keywords: Quality indicators, DPM, Sigma scale, risk mitigation

P-113
Analysis of complete blood count critical values reporting in a university hospital

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OBJECTIVES:The critical value is a result suggesting that the patient was in imminent danger unless appropriate therapy was initiated promptly. We aimed to analyze four hemogram parameters critic value reporting in a university hospital.
MATERIALS and METHODS:We retrieved critic value reporting results of hemoglobin, hematocrit, platelet, white blood cell (WBC) and neutrophil count from laboratory information system (LBS) between 01.01.2018- 30.06.2019. Critic value reporting types were classified under three headings as "there was no information because of accordance with prior result", "there was a call but nobody was reached" and "at least one staff was informed about critic value". Microsoft 2010 excel program was used.
RESULTS:There were 3305 critic value reporting in four tests; 811 WBC, 244 hemoglobin, 422 hematocrit, 1395 platelet and 433 neutrophil. 305 results reported to emergency department, 250 to outpatient, 1800 intensive care unit and 1005 to services. Distribution of three critic value reporting types were 65.2%, 10,2% and 24,6% respectively.
CONCLUSIONS:In the present study, we provide a comprehensive view of the critical value reporting process in a university hospital. All critic value reports were recorded in LBS and tried to interpret related services. The main problem is that many times laboratory staff could not reach any health staff for reporting critic value. Improving communication nets between laboratory and other hospital services and continuous education about this topic has taken an important place.
Keywords: Critic value, critic value reporting, university hospital

P-114
Unnecessary test request ratio of CA15-3 in male patients

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OBJECTIVES:Assays for the Cancer Antigen 15-3 (CA15-3) are sensitive for breast cancer, especially used to monitor patients who were undergone surgery. Because breast cancer occurs %1 of men, care must be taken to request this test. CA15-3 assays were investigated to determine the erronous test request in male patients.
MATERIALS and METHODS:CA15-3 tests which were analyzed by chemiluminescence method on Advia Centaur XPT (Siemens) analyzer were investigated over a 6-month period (from January to July 2019) from laboratory information system (ALIS, Ventura). Patients were selected according to reference range (0 - 32 U/mL) and gender.
RESULTS:A total of 6,487 CA15-3 test requests were in 6 months. 895 test requests were carried on for male patients (13.8%). According to reference range, although it was found that 832 (93%) test request were within the range, the only 63 (7%) test results were observed to be higher than reference range (median: 42, min: 33, max: 179). It was observed that the most unintelligible CA15-3 requests were from Internal Medicine (38.8%) and General Surgery (12.4%) clinics.
CONCLUSIONS:Unnecessary tests cause the laboratory workload and high costs. The use of tumor markers for screening purposes in patients with no complaints is one of the most common reasons for unnecessary test ordering. The fact that CA15-3 test can be ordered from various departments in hostitals causes the unnecessary initial test requests. Department-based test ordering restrictions and displaying a warning message during the CA15-3 test request through the hospital information system may decrease the unnecessary test ordering.
Keywords: CA15-3, breast cancer, unnecessary test request

P-115
Improvement of postanalytical phase management with an algorithm based autoverification system

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OBJECTIVES:In recent years, the number of patients admitted to hospitals and tests performed in laboratories has increased,so the workload.This may increase the likelihood of errors.There is a need for an approach to support the clinical biochemistry specialist,as well as to improve turnaround time(TAT).In large-scale laboratories, a method is required to ensure standardization of verification and to prevent errors that may occur during the verification of thousands of results. In our study,a significant proportion of results planned to verify via middleware according to rules and algorithms established by a clinical biochemist.It is aimed to improve quality and speed(TAT) and devote more time focus on results require interpretation.
MATERIALS and METHODS:The study was carried out in Gazi University Faculty of Medicine Central Biochemistry Laboratory. 22 biochemistry tests on AU5800 autoanalyzer(Beckman Coulter) autoverified by middleware program(Remisol,Beckman Coulter) in cooperation with LIS(Nucleus,Monad). Autoverification algorithms prepared by clinical biochemists consisted of QC,critical values, serum indices, autoanalyzer flags, measurement intervals, related tests, relationship of delta check value with RCV and validation range steps. Validation of the autoverification system was performed with simulated and real patients' samples. Performance of the system evaluated daily and weekly.
RESULTS:Performance of the autoverification evaluated.Test and tube-based autoverification rates were 81% and 27%,respectively.Thanks to autoverification,TAT of 22 tests improved approximately 12 minutes.As a lean approach,the status of the system can be monitored online with a dashboard in laboratory.
CONCLUSIONS:Consequently, standardization of verification,early detection of analytical errors,shortening of TAT and concentration of laboratory specialist on more important results were achieved by means of autoverification system.
Keywords: Autoverification, postanalytical phase, middleware

P-116
Calculation of measurement uncertainty of biochemical parameters and interpretation

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OBJECTIVES: The laboratories help the clinician to make the right decision with the results of the analysis. Responsibility is very important as the results to be reported will affect the patient and the clinician positively or negatively. Therefore, the main task of medical laboratories is to produce quality, accurate, reproducible results and to report on time. However, the analytical results that we assume are not always accurate. The definition of uncertainty according to the VIM (International Vocabulary of Basic and General Terms in Metrology); It is the parameter that characterizes the distribution of the values that are included with the measurement result and which can reasonably correspond to the measured size. Measurement uncertainty is a parameter that occurs during the measurement procedure and includes

factors that affect the measurement result, and the measurement uncertainty must be included with any measurement result actually obtained.
MATERIALS and METHODS: We used Cobas 8000 autoanalyzer system for this report. We worked on emergency markers which are more needed at ER and used some formulas about uncertainty of measurement, based on GUM.
RESULTS:We compared the results that we obtained and determined measurement uncertainty of each test. And with this report, we can help the clinician to make the right decision with the results of the analysis.
CONCLUSIONS: Accordingly, the reported measurement result should be the sum of the measurement value and the measurement uncertainty (1).
Keywords: Laboratory, uncertainty, measurement, accuracy

P-117
The importance of the allowable total error (TEa) target in evaluating quality of clinical chemistry

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OBJECTIVES:Different quality specifications have been defined by different organizations in various countries for clinical chemistry tests. The aim of this study was to evaluate the total error in our laboratory according to the defined allowable total error (TEa) targets.
MATERIALS and METHODS:Monthly deviations for creatinine and glucose were obtained from external quality assessment data of our laboratory for twelve months (July 2018- June 2019). In this period, the total error and six-sigma values of the laboratory were calculated for each test according to the different target TEa values offered by the quality specification programs.
RESULTS:Our total error of creatinine calculated by desirable biological variation (dBV) (Tea:8.87%) and CLIA2019 (Tea:10%) targets were higher than TEa for ten and eleven months, respectively. Calculated by CLIA (Tea:15%), RiliBak (Tea:20%) and Turkey (20%) targets, total errors for creatinine were mostly smaller than TEa. Total error of glucose calculated by dBV (6.96%) and CLIA2019 (8%) targets were smaller than TEa for six and ten months, respectively. According to CLIA (10%), Turkey (11%) and RiliBak (15%) programs, our laboratory total errors of glucose were smaller than their target TEas for all months. Sigmametric evaluation for two tests were in accordance with these results.
CONCLUSIONS:Inconsistent TEa targets from different sources causes difficulty and confusion in evaluating the laboratory quality control. The international biochemistry community need to agree on a single TEa target values for each analyte.
Keywords: total allowable error, quality specifications, glucose, creatinine, quality requirements

P-118
Low serum paraoxonase-1 level and increased risk of atherosclerosis in individuals with AB blood group

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OBJECTIVES:Although today several studies have investigated and confirmed the existence of an association between ABO blood phenotype with atherosclerosis. However the present study, according to the best of our knowledge, is the first study that focuses on apparently healthy men blood donors and investigating a relationship between AB blood group and the serum paraoxonase (PON1).
MATERIALS and METHODS:This study was conducted with one hundred and eighty-eight apparently healthy male blood donors. Laboratory test included assessment of ABO blood typing, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TGs) and serum PON1 concentrations.
RESULTS:The most essential finding was the identification subjects of significantly lower values of PON1 ($p<0, 01$) and higher values atherogenic plasma index (AIP); log10 (TG/HDL-C) ratio ($p<0, 05$) in blood group AB phenotype compared with those with non-AB blood phenotypes.
CONCLUSIONS:Especially statistically significant association between AB blood phenotype PON1 and AIP levels supports its potential role of novel atherogenic risk parameters in the pathogenesis of atherosclerosis and the clinical observations of tendency to cardiovascular disease of individuals with non-O blood groups.
Keywords: ABO phenotyping, atherosclerosis, atherogenic indices, paraoxonase, atherogenic index

P-119
Lipid status in newborn population

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OBJECTIVES:Values of certain biochemical parameters must be interpreted in relation with values for given population. Many factors influence biochemical parameters of newborn, especially on lipid status. One of them is mother lipid status.
MATERIALS and METHODS:Lipid status were measured by Roche (Cobas c501) and Abbott (Architect c8000) in 372 newborns, aged 1-5 days in Center of Clinical Laboratory Diagnostic, Clinical Center of Montenegro. Serum samples were obtained between 7 and 13 h. IBM SPPS ver. 21 program was used for statistical analysis.
RESULTS:Samples were devided into five groups (from first to fifth day). First group: Cholesterol: (median: 1.75; Iq: 1.41-2.27), Triglycerides: (median: 1.11; Iq: 0.96-1.40), HDLc: (median: 0.80; Iq: 0.60-1.06), LDLc: (mean value: 0.45±0.41). Second group: Cholesterol: (mean value: 1.95±0.64), Triglycerides: (median: 1.61; Iq: 1.34-1.93), HDLc: (mean value: 0.78±0.25), LDLc: (median: 0.42; Iq: 0.27-0.70). Third group: Cholesterol: (mean value: 2.22±0.54), Triglycerides: (median: 1.86; Iq: 1.47-2.26), HDLc: (median: 0.77; Iq: 0.58-0.93), LDLc: (mean value: 0.64±0.34). Fourth group: Cholesterol: (mean value: 2.70±0.65), Triglycerides: (median: 1.85; Iq: 1.49-2.61), HDLc: (median: 0.80; Iq: 0.65-1.09), LDLc: (mean value: 0.90±0.44). Fifth group:: Cholesterol: (mean value: 2.90±0.70), Triglycerides: (median: 1.80; Iq: 1.48-2.43), HDLc: (mean value: 1.14±0.43), LDLc: (mean value: 1.03±0.60). Statistically significante was evidenced for tested parameters between each of groups by ANOVA test, level $p<0.001$.
CONCLUSIONS:All parameters of lipid status in fifth group were statisticaly higer than in other groups. The reason for this was either samples which were delivered in laboratory in diferent time, or physiological changes which hapened by newborn growth.
Keywords: lipid status, newborn, population

P-120
Increased oxidized LDL level in individuals with a blood group

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OBJECTIVES:The ABO blood group has been associated with risk of cardiovascular disease and risk of cancer in observational studies. Also elevated serum Oxidized cholesterol -rich low -density lipoprotein (OxLDL) has been positively associated with increased risk of various types of cancer and atherosclerotic diseases. Relevantly, a prominent feature related to dysregulated lipid metabolism and inflammation is the increased production of OxLDL, which results from elevated oxidative stress. However, the effect of ABO blood group has never been studied in subjects affected by dysregulated oxidative lipid modifications.
MATERIALS and METHODS:In the present study, total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol, OxLDL concentrations were evaluated in one hundred eighty eight 188 apparently healthy men medical staff blood donors

aged from 18 to 58 years and the association between these variables and ABO blood groups was examined.
RESULTS:In the population studied we did not find any association between cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol and ABO blood groups while OxLDL levels were higher in individuals with A antigen than in subjects without this antigen(p<0,001).
CONCLUSIONS:Our data has findings that support previous studies showing that individuals with Group A may be more prone to atherosclerotic diseases.
Keywords: ABO phenotyping, oxLDL, cancer, atherosclerosis

P-121
Effect of bariatric surgery-induced weight loss on HDL, ApoA1 and OxLDL levels in six months

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OBJECTIVES:Today, bariatric surgery is very common. High-density lipoprotein cholesterol (HDL-C) amount and HDL function are very important for atheroprotection. Obese patients with metabolic syndrome have significantly reduced HDL-C levels and are often at increased risk for atherosclerotic diseases. Although weight loss benefits these patients, its effects on the change in HDL quantity and its functionality are currently poorly studied. We investigated how rapid weight loss affects HDL values and its antioxidant potential in patients undergoing a malabsorptive bariatric procedure.
MATERIALS and METHODS: Fasting plasma samples were collected from 30 morbidly obese patients with body mass index >40 one day before and 6 months after bariatric procedure, then HDL, ApoA1 and oxidized LDL (OxLDL) were analyzed using biochemical techniques.
RESULTS: The amount of OxLDL decreased dramatically after the surgery (p=0,01) and we observed a statistically significant increase in HDL concentration (+16%, P=0.0025). ApoA1 levels entered a post-operative upward trend, but a significant increase was seen in six months (p<0,05).
CONCLUSIONS: Rapid weight loss shows significant improvement in HDL concentrations and functionality, which may contribute to the anti-atherosclerotic effect of malabsorptive bariatric procedures. In addition to these findings, the decrease in oxLDL might suggest that bariatric surgery has made a positive contribution to the antioxidantive effect of HDL.
Keywords: Bariatric surgery, Paraoxonase, High-density lipoprotein cholesterol, OxLDL

P-122
Profiles of oxidative/nitrosative stress-related microRNA and mRNA expression in patients with vitiligo

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OBJECTIVES:Oxidative/nitrosative stress has a critical role in the pathogenesis of vitiligo. However, the specific molecular mechanism involved in oxidative/nitrosative stress-induced melanocyte death is not well characterized. Furthermore, little is known about the impact of oxidative/nitrosative stress on the expression of miRNAs and their targeted mRNAs in patients with vitiligo.
MATERIALS and METHODS:Vitiligo patients and age- and sex-matched controls subjects were enrolled in this study. 34 different miRNAs in plasma samples were studied. These miRNAs were evaluated using high throughput quantitative real-time PCR. Furthermore, the activities of erythrocyte catalase (CAT) and superoxide dismutase (SOD), and the levels of plasma malondialdehyde (MDA) were determined on spectrophotometer. Also, 3-nitrotyrosine (3-NTx) and nitric oxide (NO) levels in plasma as nitrosative stress biomarkers were measured measured by ELISA.
RESULTS:The results of study demonstrated that the expression level of miR-373-3p, miR-25-3p, miR-34a-5p, miR-193a-5p and miR-196a-5p was significantly upregulated in patients when compared with the control (p<0.05). The expression level of miR-2b-5p, miR-223-3p, miR-23a-3p, miR-423-5p, miR-92a-3p and miR-156-5p was significantly downregulated in patients (p<0.05). In addition, expression of 23 miRNA had upregulated or downregulated, but not statistically significantly different when compared with the control group. Besides, MDA,NO and 3-NTx levels in plasma were significantly higher, and SOD and CAT activities were significantly lower, in patients compared with controls.
CONCLUSIONS:Our results suggest that plasma miRNA levels may alter in Vitiligo and, some miRNAs and oxidative/nitrosative stress may an important the role in pathogenesis this disease.
Keywords: Vitiligo, miRNAs, oxidative/nitrosative stress

P-123
Determination of rs41507953 polymorphism in abdominal aortic aneurysm

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OBJECTIVES:Epoxyeicosatrienoic acids (EETs), a cytochrome P450 epoxygenase metabolite of arachidonic acid, have a role in ion transport and have vasodilator, anti-inflammatory as well as pro-fibrinolytic properties. The soluble epoxide hydrolase (sEH) enzyme encoded by the EPHX2 gene that converts EETs into less bioactive diols. It was demonstrated that inhibition of sEH, exhibit a protective effect on animal models of many cardiovascular diseases, include also abdominal aortic aneurysm (AAA). rs41507953 polymorphism in the EPHX2 gene that cause an increase in sEH activity have been associated with developing coronary artery disease, ischemic stroke. However, it remains unknown whether rs41507953 polymorphism are associated with AAA. Therefore, the objective of this study is to evaluate the association between AAA and EPHX2 rs41507953 polymorphism.
MATERIALS and METHODS:In this study, rs41507953 polymorphism was determined in 50 healthy and 50 AAA patients. Genotyping of EPHX2 rs41507953 polymorphism was performed by the real-time PCR using double-dye hydrolysis probes.
RESULTS:Although we found that development of AAA risk in individuals carrying heterozygous genotype for rs41507953 polymorphism was found to be 1.78 times higher than individuals carrying wild-type allele, this result failed to reach statistical significance
CONCLUSIONS:In conclusion, although heterozygous individuals have 1.78 times higher risk ratio for AAA development, statistical results showed that there was no association between the EPHX2 rs41507953 polymorphism and AAA in the Turkish population. However, further studies are needed to evaluate the association of this polymorphism and AAA in various populations which include more individuals and / or of different origins.
Keywords: Epoxyeicosatrienoic acids, abdominal aortic aneurysm, soluble epoxide hydrolase

P-124
Comparison of antioxidant properties and phenolic contents of zucchini and potato according to consumption methods

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OBJECTIVES:There is evidence that increasing consumption of vegetables reduces the risk of certain chronic diseases such as hypertension, stroke, and cardiovascular diseases partly as a result of consumption of antioxidant substances. In this study, it was aimed to investigate the effects of zucchini and potato consumption methods on their antioxidant activity (AA) and total phenolic content.
MATERIALS and METHODS:Zucchini and potato were homogenized in distilled water at concentration of 10 g/dL. Raw group (n=10) was stored in the refrigerator at +4°C, frozen group (n=10) was stored in freezer at -20°C and cooked group (n=10) was heated in oven at 150°C for 20 minutes. Phenolic substance amount was determined by using Folin-Ciocalteu reagent and AA was determined by detecting 2,2-diphenyl-1-picrylhydryl (DPPH) radical scavenging activity. Results were given as mean±SD.
RESULTS:The phenolic content and AA values (DPPH%) of potato were compared among the groups; AA values of the raw group (3.01±0.52%) were significantly higher than frozen (0.44±0.28%; $p<0.001$) and cooked group (2.06±0.84%; $p<0.05$). Moreover, the phenolic content of raw group (3.78±0.15mg/dl) was significantly higher than frozen (2.84±0.18 mg/dl) and cooked group (1.88±0.17 mg/dl) ($p<0.001$ for both). For zucchini, it was found that the AA values were significantly higher in cooked group (2.92±0.46%) than raw (0.14±0.55%) and frozen group (1.07±0.49%) ($p<0.001$ for both). Additionally, the cooked group (2.70±0.08 mg/dl) had significantly higher phenolic content than raw (1.64±0.06 mg/dl) and frozen group (2.10±0.14 mg/dl) ($p<0.001$ for both).
CONCLUSIONS:Findings of this study might be used to increase the beneficial effects of vegetables according to the consumption methods.
Keywords: antioxidant, cooking methods, phenolic compounds, potato, zucchini

P-125
The evaluation of serum vitamin B12 levels at Sanliurfa city

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OBJECTIVES:Vitamin B12 (cobalamin) is a water-soluble vitamin that plays essential roles in red blood cell formation, cell metabolism, nerve function and the production of DNA. Vitamin B-12 deficiency can lead to anemia, fatigue, muscle weakness, intestinal problems, nerve damage and mood disturbances. The aim of this study was to investigate serum vitamin B12 levels in Sanliurfa city.
MATERIALS and METHODS:Serum vitamin B12 levels of 4022 patients were evaluated. The patients who had admitted to Harran University Hospital between June 31-July 31 2019 were retrospectively screened. Serum B12 levels <100 ng/ml is accepted as deficiency, 100-400 ng/ml is moderate and >400 ng/ml sufficient.
RESULTS:The mean B12 levels were 74.73±19.15 ng/ml in 15 patients (0.38%) that referred to deficiency (<100 ng/ml), 271±64.24 ng/ml 3028 in patients (75,28%) referred to moderate (100-400 ng/ml) and 484.8±64,94 ng/ml in 979 in patients (24,34%) referred to sufficiency (>400 ng/ml).
CONCLUSIONS:In this study, in patients who admitted to our hospital Sanliurfa city, no serious vitamin B12 deficiency was detected and in most patients th elevels were found moderate. Vitamin B12 levels vary according to region and nutritional conditions in different age groups and gender.
Keywords: Sanlıurfa, Vitamin B12, Prevalence, Reference Range

P-126
The effects of prolonged fasting model on energy metabolism and mitochondrial functions in neuronal

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OBJECTIVES:It is known that long-term fasting (IF) model in humans can reduce inflammation and severity of chronic diseases, delay aging and increase health. The most important finding known is that the body is exposed to abundant ketone bodies as a result of fat destruction during prolonged fasting. In this study, we aimed to investigate the changes in energy metabolism in neuron cell cultures and the contribution of ketone bodies in these changes.
MATERIALS and METHODS:SH-SY5Y (human neuroblastoma-ATCC / CRL 2266) cells were used in the project.Cells were incubated for 16 hours with normal diet, calorie restriction media, fasting model (glucose reduced blood) and also non-glucose medium.Ketone was added to the other flasks containing the same media simultaneously and mitochondrial functions were evaluated in the cells while lactate, lactate dehydrogenase, ketone and glucose levels were measured in the media to show changes in the energy metabolism of all cells. Mitochondrial functions were determined by performing citrate synthase activity and flow cytometry measurements.
RESULTS:The results obtained from repeating experiments have shown us that the cells use ketones,regardless of the amount of glucose, especially in the ketone-added models.There were positive changes in mitochondrial functions of ketone added cells.When ketones were added, especially in the models with fasting model,the increase in membrane potential and flow cytometry activity were observed.
CONCLUSIONS:With these findings, we think that the presence of ketone in cell mediums has a great contribution to neuron cell energy metabolism and it may be beneficial to use exogenous ketone treatment in the treatment of neurological diseases.
Keywords: Neuron Cells, Fasting, Keton Bodies, Mitochondrial Function

P-127
Antioxidant and antimicrobial activity of einkorn (Triticum monococcum L)

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OBJECTIVES:Wheat is a highly important cultivation plant used to meet a large part of our nutritional needs. Demand for wheat as food is increasing all over the world, including countries whose climates are not suitable for growing wheat.Wheat, which is one of these cultivated plants, is black wheat. ‘Siyez’ or ‘spa’ are the local names of einkorn in Turkey. It is known that black wheat (Triticum monococcum spp. Monococcum), which is one of the ancestors of wheat (Triticum ssp.), Contributes to human nutrition and health.High protein, carotenoid and tocol content of black wheat (einkorn) and lower toxicity than other Triticum species. Therefore, we aimed to determine the antioxidant and antimicrobial activity of einkorn (Triticum monococcum L) extracts.
MATERIALS and METHODS:The einkorn wheat was obtained from, Kastamonu (Turkey). The determination of antimicrobial activity of einkorn extracts against Pseudomonas aeroginosa (ATCC 27853), Escherichia coli (ATCC 25922), Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 25923) and Candida albicans (ATCC 10231) were investigated by disc and agar-well diffusion method. Radical scavenging activities of einkorn extracted in solvent was measured via spectrophotometric methods.
RESULTS:As a result, it was determined that water extracts of siyez wheat had high free radical scavenging effects. In this study, it was found that wheat extracts used showed various degrees of antimicrobial activity against microorganisms tested.
CONCLUSIONS:Due to its rich content and high biological activity, it is thought that it should be developed in modern healthy wheat varieties and included more effectively in the nutrition process.
Keywords: Triticum monococcum L,Antimicrobial activity,Antioxidant activity

P-128

Adropin and orexin levels in new diagnosed obstructive sleep apnea syndrome patients

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OBJECTIVES:Obstructive sleep apnea syndrome (OSAS) is associated with repeated episodes of upper airway obstruction during sleep. Obstruction of the upper airway can lead to a decrease in blood oxygenation which is mainly associated with metabolic diseases. Orexin is a neuropeptide, which is important in the regulation of eating behavior and sleep. Adropin is a small peptide encoded by energy homeostasis associated gene. In this study we hypothesized that orexin and adropin levels are changed during sleep in OSAS patients. The aim of this study was to determine circulating orexin and adropin levels in newly diagnosed OSAS patients.
MATERIALS and METHODS:OSAS patients (n=7) and age/gender matched healthy subjects (n=30) are added in the study. After polysomnographic recording whole blood were collected. Adropin and orexin levels were determined using commercial available ELISA kits in serum samples. Blood biochemical parameters and PSG results are correlated. Results are given as mean±SD and p<0.05 were identified as significant.
RESULTS:Our results show that apnea/hypopnea index(AHI) was significantly higher in OSAS patients (28,5±21,8 vs. 1,9±0,9;p<0.001). Orexin levels were significantly reduced in patients with OSAS(643,8±239,8 vs. 1217,7±701,9 pg/ml;p<0.001) but adropin levels (1205,4±232,2 vs.1269,6±181,3 pg/ml;p=0,2199) were not changed, with respect to the control group. CRP,triglyceride, total cholesterol and LDL-cholesterol levels are significantly higher in OSAS patients, because HDL-cholesterol, total lipid and fasting glucose levels were not changed.
CONCLUSIONS:In conclusion, our results show that orexin levels are significantly associated with AHI in OSAS patients, as expected. Orexin is the major neuropeptide that regulates the metabolism and sleep pattern is OSAS patients.
Keywords: Obstructive sleep apnea syndrome, adropin, orexin, polysomnography.

P-129

A diagnostic algorithm for assessment of liver fibrosis

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OBJECTIVES:Liver fibrosis (LF) affects between 4.5 and 9.5% of the world population. The liver biopsy still is the golden standard for the diagnosis of the LF, but it is invasive, requires trained personnel, and carries the risk of adverse reactions. Thus, the utility of serum fibrosis markers is under investigation for predicting changes of the LF status. Our study aimed to evaluate the pertinence of the eLIFT (easy Liver Fibrosis Test) algorithm for non-invasive assessment of liver fibrosis and cirrhosis in patients with confirmed Chronic Hepatitis B infection or (ALD) alcoholic liver disease; also to compare the diagnostic significance of the eLIFT with other commonly used serum biomarkers, such as AAR, APRI, GPRI, Fib-4, ELF.
MATERIALS and METHODS:The investigation was conducted with 100 healthy controls, 150 patients with HBV, and 50 patients with ALD. All participants were tested for the above stated parameters. The combined mathematical equations with direct and indirect markers are considered more reliable.
RESULTS:The results for sensitivity and specificity: a) AAR - 81.3% and 55.3%; b) APRI and GPRI show similar results - approximately 70% and 65% respectively; c) Fib-4 - 97% and 65%; d) ELF algorithm for moderate LF 69% and 98%, for cirrhosis – 83% and 97% respectively.
CONCLUSIONS:eLIFT is appropriate for advances and for mild LF diagnosis, thus it is appropriate for the first line of testing for LF. It is convenient test because it is easily accessible and reasonably costly and shows acceptable sensitivity and specificity for ADL and HBV.
Keywords: eLIFT, Liver Fibrosis

P-132

Anti-phospholipase A_2 receptor antibodies in the diagnosis of primary membranous nephropathy

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OBJECTIVES:Two forms of membranous nephropathy (MN) have been described - the primary form (PMN) and the secondary form (SMN). It is believed that phospholipase A2 receptor (PLA2R1) is a target autoantigen in about 80% of patients with MN. The aim of our study was to compare the levels of anti-phospholipase A2 receptor antibodies (anti-PLA2R1) in patients with PMN, SMN, others glomerulonephritis (OGN) and healthy controls (HC).
MATERIALS and METHODS:The study included patients with PMN (n = 52), SMN (n = 12), OGN (n = 49) and HC (n = 50). The serum concentration of anti-PLA2R1 was determined with ELISA kit (Anti-PLA2R ELISA, IgG, EUROIMMUN, Lübeck, Germany) using MR-96A microplate reader (MINDRAY). All data are presented as mean ± SD. Significance was defined as P < 0.05.
RESULTS:The groups did not differ significantly in mean age (P = 0,055) and gender (P = 0,872). There was significant difference in mean anti-PLA2R1 concentrations between groups (P < 0.0001). The mean anti-PLA2R1 concentration of patients with PMN was significantly higher than the HC (213.97 ± 588.69 RU/ml vs 5.32 ± 3.91 RU/ml, P = 0.00l). There was no difference in anti-PLA2R1 between SMN patients and HC (6.34 ± 11.68 RU/ml vs 5.32 ± 3.91 RU/ml, P =0.193). OGN patients showed lower anti-PLA2R1 than the HC (3.52 ± 3.91 RU/ml vs 5.32±3.91 RU/ml, P = 0.002).
CONCLUSIONS:Our data suggest that anti-PLA2R1 shows a significant elevation in PMN patients and may be used as a diagnostic biomarker.
Keywords: membranous nephropathy, anti-phospholipase receptor antibodies

P-133

Application of UV light and temperature period of biosensors developed for determination of serum iron

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OBJECTIVES:Enzyme-based chemical biosensors are based on biological recognition. Temperature and UV light are important factors affecting the balance of enzymes and the rate of enzymatic reactions. In this study, optimum temperature and UV light duration were investigated in biosensors developed for the determination of iron in serum.
MATERIALS and METHODS:The bioactive layer was prepared by immobilizing the hydrogen peroxidase enzymes on the gold electrode with UV light using bovine serum albumin (BSA), gelatin and glutaraldehyde. Measurements were obtained using acetate buffer (10mM, pH 6.0) with electrodes immobilized by applying UV light for 30, 40, 50, 60 and 70 minutes. Measurements were performed at 30 °C, 35 °C, 40 °C and 45 °C with the electrode prepared using 40 min uv light time to measure the optimum temperature.
RESULTS:In this study, the best measurement was obtained with electrode applied to the bioactive layer with a UV light time of 40 minutes and under operating conditions where the temperature was 40 °C.
CONCLUSIONS:For biosensors prepared with bioactive layer hydrogen peroxidase enzyme, we can recommend 40 minutes UV light time and 40 minutes temperature for optimum working conditions.
Keywords: biosensor, hydrogen perxidase, optimization

P-134
Serum ghrelin levels in bipolar disorder patients with metabolic syndrome treated by valproic acid

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OBJECTIVES:Metabolic syndrome (MS) appears to be much more common in patients with bipolar disorder (BPD) than in the general population. In the treatment of BPD, valproic acid (VPA) is one of the commonly used pharmacological agents. In many studies, it has been reported that the levels of appetite-enhancing ghrelin are related to MS. The aim of this study is to assess the effect of VPA on ghrelin levels in patients with MS and BPD.
MATERIALS and METHODS:40 BPD patients with VPA treatment and 20 healthy controls were included in the study. BPD patients were divided into 2 groups: 1. BPD patients with MS, 2. BPD patients without MS. The BPD patients diagnosed according to the Diagnostic and Statistical Manual for Mental Disorders (DSM IV). The MS diagnosis was based on the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III) criteria. Serum ghrelin levels of control and patient groups were determined spectrophotometrically according to ELISA method.
RESULTS:Serum ghrelin levels were significantly lower in BPD patients with MS compared to BPD patients without MS and control group ($p < 0.001$).
CONCLUSIONS:These results indicate that serum levels of ghrelin and adiponectin are related to MS, but VPA therapy does not affect the results of the ghrelin.
Keywords: Metabolic syndrom, bipolar disorder, valproic acid, ghrelin.

P-135
Evaluation of clinical use habits of tumor marker tests

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OBJECTIVES:Tumor markers (TMs) result from the re-expression of substances by embryologically related tissues. Many are found in different tumors of the same tissue. Therefore, they have low specificity and are not sufficiently sensitive as a screening test. The aim of this study is to evaluate the TM requesting habits of clinicians in Usak Training and Research Hospital, and the appropriateness of the test requests with the diagnosis.
MATERIALS and METHODS:Data of 6998 serum TMs requested from 3316 patients between May 1 and July 31, 2019 were obtained from Laboratory Information System and grouped as sex, age, disease diagnoses and multiple requests (more than 3 tests simultaneously). Compliance with diagnosis was evaluated as appropriate or inappropriate based on published guidelines for indications for TM requests.
RESULTS:796 of the 6998 TMs requested from inpatients (2.75 markers/patient) and 6202 from outpatients (2.04 markers/patient). Most TMs were made in the 50-70 age range (48.3%). Multiple TMs were mostly demanded from the Obstetrics and Gynecology Clinic with the diagnosis of menstrual irregularity. Also, 1078 of 1408 total PSA and 28 of 191 free PSA tests were requested with appropriate pre-diagnosis.
CONCLUSIONS:This study is an example of the use of data mining for conformity assessment purposes of the TM requests. Accordingly, it was found that the TMs were often incompatible with the diagnosis and were used for general screening purposes. In order to minimize misuse, evidence based indicators should be developed and clinician awareness should be increased by creating test request algorithms that support the diagnosis.
Keywords: Tumor marker, request, diagnosis

P-136
Evaluation of the Inflammation status and bioimpedance data in chronic hemodialysis patients

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OBJECTIVES:Chronic kidney disease (CKD) is an increasingly important health problem in the World. Inflammation and anemia is a common feature in dialysis patients and is associated with cardiovascular complications and poor outcome. Bioelectrical impedance analysis (BIA) provides a non-invasive assessment of body composition. In this study, we aimed to compare the inflammation and bioimpedance data by using Hepcidin, IL-6, TNF-α, hsCRP, sTFR, LRG parameters.
MATERIALS and METHODS: For this purpose, 74 hemodialyzed patients who applied to the nephrology outpatient clinic of Selcuk University Faculty of Medicine were included in the study. Hepcidin, IL-6, TNF-α, sTFR, LRG analysis of the remaining blood samples after the routine tests and controls of the patients were performed by ELISA and hsCRP analysis was performed nephelometric method
RESULTS:We found a close relationship between functional anemia parameters, inflammation and arterial stiffness markers, central hemodynamics and nondiping status.
CONCLUSIONS:This relationship should be evaluated for routine availability in the larger patient group.
Keywords: Hepcidin, IL-6, sTFR, LRG, hsCRP and bioimpedance

P-137
Is plasma always a suitable alternative to serum in biochemical analysis?

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OBJECTIVES:Serum is the most widely used sample in biochemical analysis, but plasma has some advantages when compared to serum such as reduction in turnaround time, no fibrin and gel-based interference and more accurate reflection of the in vivo situation. The aim of this study was to evaluate whether there is a difference between serum and plasma for 20 analytes.
MATERIALS and METHODS:Total of 50 healthy subjects were included in the study. Blood samples were collected in tubes with gel (BD) and containing lithium heparin (BD, 4.0 mL). Samples in tubes with gel were allowed to clot at room temperature. Serum and plasma were obtained by centrifugation at 2000 g for 10 minutes. Hemolysis index was lower than 30 in serum and plasma samples. Biochemical measurements for 20 analytes were performed within two hours on Cobas c702 (Roche Diagnostics GmbH, Mannheim, Germany).
RESULTS:Lactate dehydrogenase (LDH) activity, potassium and phosphate levels were higher ($p<0.001$) although total protein was lower ($p<0.001$) in serum when compared to plasma.
CONCLUSIONS:The use of serum reference ranges is not suitable for plasma LDH, potassium, phosphate and total protein measurements. Plasma is a better quality sample because it is independent of fibrin and gel interference. Each laboratory may prefer serum or plasma according to their test panel.
Keywords: Serum, plasma, biochemical analysis

P-138
Ph optimization for a new urea biosensor

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OBJECTIVES:Urea is a harmful substance that is formed as a result of the use and breakdown of protein foods. This substance is excreted in the form of

urine by draining by the kidneys. If the kidneys cannot remove this substance sufficiently, they begin to accumulate in the blood. Its elevation has a toxic effect on the body, and when it is too high it is impossible to live. Because of these reasons, urea determination is of great medical importance.Enzymes are not very resistant to strong acids and bases.Therefore, the determination of the pH in the enzyme studies is very important.
MATERIALS and METHODS:. In this study, we aimed to design a new amperometric biosensor for urea determination.In this study for the determination of Urea, urease enzyme was immobilized on the graphite electrode by using BSA/gelatin and crosslinking by glutaraldehyde. Measurements were carry out at 0.2 V. Optimization studies of the designed biosensor were carried out first for the bioactive layer components and ph optimization.
RESULTS:From the bioactive layer optimization studies; gelatin, bovine serum albumin amount and optimal percentage glutaraldehyde were determined as 0.45 gr, 0.030 gr and %2.5 for the Graphite/BSA- Gelatin/ Urease /glutaraldehyde modified biosensör. Ph 5 was found in 100 mM acetate buffer
CONCLUSIONS:As a result, it is recommended as the optimum ph 5 for the designed biosensor.
Keywords: Urea biosensor, urease, optimization

P-139
The local clinical validation of different brands of blood collection tubes for complete blood count

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OBJECTIVES:In addition to the product quality and its validation, cost-effectiveness is also important in the blood collection tube (BCT) selection. Thus, a manufacturer may prefer to add a more cost-effective BCT option to its portfolio without compromising quality and may offer different options to a customer at once. However, when a laboratory administrator needs to select a BCT or to replace, local validation of BCT should be met first. We aimed to ensure local clinical validation of different brands of BCTs, including a new tube in the market for complete blood count (CBC).
MATERIALS and METHODS:Venous blood samples were taken from 40 inpatients and were collected in four different brand evacuated tubes with K2EDTA (Vacutainer;Becton, Dickinson and Company,USA) (S-Monovette;Sarstedt Ag & Co. KG,Germany) (Vacuette;Greiner Bio-One GmbH,Austria) (Samplix;Greiner Bio-One GmbH,Austria). White blood cell(WBC), red blood cell(RBC), hemoglobin, platelet(PLT) were analyzed using a CBC analyzer (DxH 800;Beckman Coulter Inc.,USA).
RESULTS:The Vacutainer, current BCT for CBC in routine were compared with S-Monovette, Vacuette, and Samplix and bias (%) results were calculated as follows: 1.85,-0.05, and -0.43 for WBC; 0.27,-0.11, and -0.39 for RBC; 0.07, -0.07, and -0.33 for hemoglobin; 0.06, 0.25, and 0.53 for PLT. All bias calculations were within the desirable limits based on the Ricos' biological variation data.
CONCLUSIONS:Similar CBC results were obtained among BCTs, including Samplix, when compared to the Vacutainer, the tube in laboratory use. Before selecting or replacing a BCT tube, it must be validated locally by comparing with the tube in use, thus ensuring the sustainability of CBC results.
Keywords: Blood cell count, blood specimen collection, validation studies

P-140
Nutritional habits in children with autism and cerebral palsy and its evaluation with biochemical approaches

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OBJECTIVES:We aimed to investigate the nutritional state of children who suffer from cerebral palsy and autism.
MATERIALS and METHODS:A questionnaire was applied to 70 children with cerebral palsy and 32 children with autism who continue their education at the İller Bankası Special Education and Rehabilitation School. In total 102 students participated in the study with 21 healthy siblings as the control group.
RESULTS:The ratio of boys to girls with cerebral palsy was 1% while it was 4,3% in the autism group. 66,7 % of children with cerebral palsy were slim while 33,3% of children were with normal weight. 50 % of children with autism were overweight and obese. The ratio of epilepsy was 30% in children with cerebral palsy, while it was 21,9% in children with autism. There are studies showing that various special diet and sustenance provides positive behavioral change on children with autism. It was identified that only 4 children (3,92 %) with cerebral palsy (3) and autism (1) from the total of 102 children was following a special diet and this is a low rate. Given the detrimental effects of undernutrition on physical and cognitive development, monitoring of nutritional status is important in children with neurological disorders.
CONCLUSIONS:According to the results, the rate of people who has normal BMI amount has been found %37,5, considering children with both cerebral palsy and autism and healthy siblings. According to the clinical diagnosis; sex, additional health problems, supplemen tay food consumption, digestive system problems, the difference between the past important operation has been found significant statisticaly ($p<0,05$). While considering the harmful effect of undernourishment on both physical and mental development, following nutritional aspect of a children who has neurological disorders has quile importance. In the light of our survey we know that further researches are essential on the topic of nutrition disorders and behavioral problems of children with autism, digestive system diseases the relation between the past attacks and crisis, the prevention of undernourishment of children with celebral palsy, and the contribution of diet on the treatment of epilepsy.
Keywords: cerebral palsy, autism, nutrition

P-141
Comparison of serum hemolytic index and manual spectrophotometric measurement of free hemoglobin

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OBJECTIVES:The effect of hemolysis as a preanalytical error on laboratory test results is significant. In this study, it was aimed to evaluate hemolytic index (HI) values obtained from emergency and routine biochemistry laboratory and to compare them with those the results of a manual free hemoglobin method.
MATERIALS and METHODS:In April 2018 and 2019, serum HI values of emergency and routine biochemistry laboratory obtained from laboratory information management system were examined. One hundred of serum samples with different hemolytic index values within a wide range (25–619) were studied by Cobas 6000/8000 analysers and hemoglobin concentrations were determined by a manual spectrophotometric method. Hemoglobin levels in mg/dL were calculated by two different methods by absorbance measurements at 380, 415 and 450 nm and 415, 450 and 700 nm, Method 1 and Method 2, respectively. Regression analysis and % bias values were calculated between serum hemolytic index and manual method results.
RESULTS:HI values >50 mg/dl were 16.82%, 12.21% in emergency laboratory and 1.69%, 1.22% in routine laboratory, respectively. Serum HI showed a high correlation with Method 1 (r=0.969) and Method 2 (r=0.973). Percent bias

values were 9.43% and 8.88% for Method 1 and 2, respectively.
CONCLUSIONS:Because of the effect of hemolysis on test results, many samples may be rejected although redundant and lead to delayed patient results. This can be reduced by appropriate blood collection and training of laboratory technician and use of serum HI. Evaluation of HI by laboratory specialist may contribute to accurate clinical interpretation and reduction of sample rejection.
Keywords: Serum hemolytic index, hemolysis, preanalytical error

P-142
Comparison of vitamin D levels in different types of tubes

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OBJECTIVES:The newly introduced BD barricore plasma collection tubes provides ease of use for laboratory workers. In this study, we aimed to compare vitamin D levels in BD Vacutainer K2 EDTA tube with Vacusera gel tube and BD Vacutainer barricore plasma collection tubes.
MATERIALS and METHODS:Twenty healthy volunteers participated in the study. Venous blood samples were collected in each type of tubes in the morning. The tubes without anticoagulant was allowed to coagulate for 20 minutes. The samples were then centrifuged at 1500 g for 10 minutes. Vitamin D levels of all three samples were analyzed by HPLC. The distribution of the data were evaluated. Since the distributions were non-Gaussian, the differences between the groups were investigated by Wilcoxon test. Then, the relationships between the groups were examined by Spearman correlation test.
RESULTS:There were no statistically significant differences between BD Vacutainer K2 EDTA tube, Vacusera gel tube and BD Vacutainer barricore plasma collection tubes (p=0.911, p=0.823, respectively). BD Vacutainer K2 EDTA tube and Vacusera gel tube and BD Vacutainer barricore plasma collection tube results were well correlated with each other (r=0.892, p<0,01; r=0.920, p<0,01, respectively).
CONCLUSIONS:Although HPLC is a reliable method for vitamin D analysis, it is known that serum seperator gels may cause interference. The use of EDTA tubes is therefore recommended by the manufacturer. In our study, Vacusera gel tube and BD Vacutainer barricore plasma collection tube were compared with BD Vacutainer K2 EDTA tube and the results were found to be consistent.
Keywords: vitamin D, barricore plasma collection tube, compare

P-143
Retrospective evaluation of vitamin D, calcium and vitamin B12 levels

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OBJECTIVES:Vitamin D, calcium and vitamin B12 are known to have important effects on human health through various mechanisms. In this study, we aimed to determine whether vitamin D, vitamin B12, and calcium levels differ in terms of age and sex in hospital admissions in the last 6 months.
MATERIALS and METHODS:6087 patients who applied to Konya Training and Research Hospital between 01.01.2019-01.07.2019 were screened on the hospital information system.
RESULTS:Of the 6087 patients, 73.1% (n=4448) were female and 26.9% (n=1639) were male. The mean age of the patients was 41.19 ± 20.14 (0-96), the mean calcium level was 9.55 ± 0.44 (5,05-12,90), the mean vitamin D value was 16.33 ± 11.8 (2,10-138,95), and the mean vitamin B12 value was 382.42 ± 167.13 (103-1973). Vitamin D and calcium were significantly higher in males than females (respectively, p=0.011, p=0.000). In women, there was a positive correlation between calcium and vitamin B12, between vitamin B12 and vitamin D, and between calcium and vitamin D (respectively, p=0.082, p=0.232, p=0.091). There was a low positive correlation between vitamin B12 and vitamin D in male (p=0.141). İn addition, vitamin D was found to be lower in age 65 and over.
CONCLUSIONS:In our retrospective study, we found that vitamin D was highly insufficient in elderly people and that it was lower in women compared to men. We think that vitamin D supplementation may be beneficial in elderly and women.
Keywords: Vitamin D, calcium, vitamin B12

P-144
Evaluation of first trimester screening tests

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OBJECTIVES:We aimed to evaluate the ability of maternal serum plasma protein A (PAPP-A) and free beta-human chorionic gonadotropin (free ß-hCG) values measured in the first trimester screening test to predict complications that may develop in later gestational weeks.
MATERIALS and METHODS:The study included 3166 women between 16-46 years old of age. Their gestational ages were between 10 weeks and 6 days to 13 weeks and 6 days. They had a single live pregnancy, and no any complicated obstetric history and chronic systemic disease. The results of 3166 pregnant, applied to the Biochemistry Laboratory of Gaziosmanpasa University Faculty of Medicine between 2017 and 2019, were evaluated retrospectively. p values less than 0.05 were considered statistically significant.
RESULTS:The mean age of the patients was 27.43 ± 5.46 and their weight was 65.66 ± 13.13 kilograms. Crown rump lengthes (CRL) were determined as 60.71 ± 8.56 mm. Free ß-hCG levels were 55.1 ± 132.07 ng / mL and PAPP-A values were 3683.53 ± 2486 mIU / l. Nuchal translucency measurement (NT) was determined as 1.38 ± 0.37mm. None of patients had age risk. Only 2 patient (% 0,1) had Trisomy 18 risk while 59 patient (% 1,9) had Trisomy 21risk.
CONCLUSIONS:The accuracy and performance of 1st trimester screening tests, which are used in the diagnosis of neural tube defects and chromosomal anomalies and guide for further interventional procedures, should be improved. Measurements should be performed with strict internal and external quality programs.
Keywords: free ß-hCG, PAPP-A, Binary screening test, 1st Trimester

P-145
Modulation of monoaminergic response to the SNC active pharmacotherapy

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OBJECTIVES:The study of chemical neurotransmitters has acquired in recent years a very large scale, driven mainly by the discovery and use of methodologies and techniques for investigating more complex and more accurate. Given the pathophysiological importance of noradrenaline, dopamine, serotonin and γ-aminobutyric acid, we plan to study the effect of active drugs on the central nervous system on brain levels of these neurotransmitters.
MATERIALS and METHODS:We used white Albino Swiss mice which was randomized into seven groups treated with the following drugs: valproic acid (V), risperidone (R), fluoxetine (F), lithium (Li), and associations: V+F,V+Li,V+R. Brain tissue was collected, and were determined the concentrations of neuronal noradrenaline (NA), dopamine (DA) by HPLC, serotonin (5-HT) by LC-MS, and gamma-aminobutyric acid (GABA) was determined by a spectrofluorimetric method.
RESULTS:The administered drugs increased the noradrenaline brain concentration. Lithium was the most potent catecholaminergic stimulator of the brain concentration (406.66% effect increase) (p<0.001) between all the studied drugs. Co-administration of Li + Q

triggers, molecular mechanisms that cause a sudden decrease of the NA. The risperidone acts as an atypical antipsychotic: increasing concentrations of NA, DA, 5-HT and decrease the concentration of GABA.
CONCLUSIONS:The experimental results presented in this paper led, in addition to unique conclusions for the current scientific research, as well as potential theories and responses to multidrug resistance in various neuropsychiatric disorders.
Keywords: multidrug resistance, neurotranmitters, CNS active drugs

P-146
Comparison of Beckman Jaffe and enzymatic creatinine methods

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OBJECTIVES:Serum creatinine is measured as a kidney function test in almost all clinical laboratories.Creatinine methods used in clinical laboratories are generally based on automated chemical or enzymatic methods.Jaffe assays remain the predominant method type in most developed countries.It is stated that enzymatic method is the most compatible method with reference method.The aim of this study is to compare the analytical performance characteristics of Jaffe and enzymatic methods.
MATERIALS and METHODS:Serum specimens were collected from 107 hemodialysis patients (pre- and post-dialysis samples),50 patients with high creatinine levels from nephrology service,160 patients with normal creatinine values from other sections.Samples were measured with two original creatinine reagent kit(BECKMAN COULTER) based on different methodology (Jaffe and Enzymatic),by using AU 5800 autoanalyzer at Gazi University Medical Faculty Hospital Biochemistry Laboratory.Statistical analysis was performed with MEDCALC and SPSS for method comparison.
RESULTS:We found significant and strong correlation between the two methods. (r=0,995,p<0,0001).However,in the analysis of Deeming regression equations gave a slope of 1,0853 and an intercept of -0,06130.When we analyze the data by dividing it into normal and high values,Deeming regression equations gave a slope of 1,0638,1,0959 and an intercept of - 0,01073,-0,1455,respectively.Mean values for Jaffe first and second,enzymatic first and second measurements were 3.78mg/dL,3.86mg/dL and 4.05mg/dL, 4.11mg/dL,respectively.There was a significant difference according to T test(p <0.05).
CONCLUSIONS:Although it was seen that jaffe method gave higher results than enzymatic method in literature,we found that enzymatic method measured higher.We explain this with differences between methods. Therefore, more comprehensive studies with different patient groups and different methods are needed.
Keywords: creatinine,enzymatic,Jaffe,method comparison

P-147
Possible input of diabetes and smoking cigarettes in confirmation of AMI diagnosis

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OBJECTIVES:Influence of diabetes on development of ACS is well known, as well the active smoking that can pose a particular risk in increasing heart muscle ischemia. Several proposed models by international associations, indicate an increased risk and confirmation of the definition of myocardial infarction (MI) with presence of diabetes and cigarettes smoking habit in patients.
MATERIALS and METHODS:Our study included 200 patients admitted to the emergency department with symptoms of AMI. Patients samples were submitted for CK, CKMB, TnT, TnI, Myoglobin determination and estimation for presence of diabetes and smoking habit. The obtained data were compared and statistically processed versus group of patients without any of risk factors.
RESULTS:We found that 34% of patients has stable/unstable angina, and 49% was diagnosed as MI. Higher percentage of diabetic patients 28.8% has MI compared to 13.4% in patients with angina pectoris. In terms of smoking as a risk factor, 54.6% of patients with MI were active smokers compared to 34.3% in patients with angina pectoris. At diabetic patients MI was confirm with significant upper CK activity (65%), CKMB (56.8%) and TnT concentration (142%). Regarding the smokers, the most significant change was found in higher CK activity (60%) and myoglobin concentration (127.3%) in patients with AMI.
CONCLUSIONS:Result shows that those two risk factors can afford valuable data in primary diagnosis along with some sensitive but not most specific parameters such CK and Myoglobin. This attitude is based concerning their effects on metabolic oxygen supply of heart muscle.
Keywords: risk factors,myocardial infarction, cardiac markes.

P-148
Determination of adropin, desnutrin and glucagon like peptid-1 levels in emphysema disease

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OBJECTIVES: Emphysema; pathologically, alveoli is a lung disease with expansions, alveolar wall destruction and irreversible alveolar losses if filled with excess air. Pathological factors such as inflammatory cell response, protease/antiprotease imbalance, oxidative stress, apoptosis, and regeneration dysfunction in alveolar epithelium due to environmental or genetic factors play a role in the development of the disease. Dyspnea, cough, activity restriction as the disease progresses, loss of appetite and intense weight loss are the most obvious symptoms. The aim of this study was to measure the serum levels of Adropin, Desnutrin (ATGL) and Glucagon-like peptide-1 (GLP-1), which have important effects on carbohydrate and lipid metabolism of the patient and control groups, and to determine the effects of these parameters on the diagnosis and treatment of emphysema
MATERIALS and METHODS: The study group consisted of 35 patients diagnosed with Emphysema and 35 healthy subjects.
RESULTS: The study group consisted of 35 patients diagnosed with Emphysema and 35 healthy subjects. As a result of experimental analysis Adropin (p=0,002), Desnutrin (p=0,001) and GLP-1 (p=0,006) levels were compared statistically; the difference between the patient and control groups was significant (p<0,05).
CONCLUSIONS: In conclusion, in our study, it was determined that serum Adropin, Desnutrin and GLP-1 levels decreased significantly in patients with emphysema. Therefore, we believe that these parameters will contribute significantly to the diagnosis and treatment of emphysema. We think that the data obtained will shed light on the more comprehensive research for the treatment of emphysema.
Keywords: Emphysema, Adropin, Desnutrin, Glucagon like peptide-1

P-150
Oxidative status in patients with nonsyndromic cleft lip with/without cleft palate

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OBJECTIVES:Nonsyndromic cleft lip with/without cleft palate (NSCL/P) is one of the most common human congenital defects. Reactive oxygen species and oxidative stress act as teratogenic agents, leading, during embryogenesis, to several structural changes in the developing fetus. Numerous reports have described free radical-mediated congenital defects. The aim of this paper is to

determine the oxidative status in patients with cleft lip and/or palate.
MATERIALS and METHODS:Patients with NSCL/P (n = 12) and age- and sex-matched healthy control subjects (n = 13) were enrolled in this study. Malondialdehyde (MDA) concentrations as oxidative stress biomarker in plasma, and the activities of superoxide dismutase (SOD) and catalase (CAT) as antioxidant enzymes in erythrocyte were determined as spectrophotometric.
RESULTS: Oxidative stress was confirmed by the significant elevation in MDA concentrations ($p<0.05$). Besides, increased CAT and SOD activities were found in patients with NSCL/P compared with the control group ($p<0.05$).
CONCLUSIONS: Our findings indicated that increased the antioxidant enzyme activities and MDA concentrations in patients with NSCL/P may be an adaptative response to against oxidative stress.
Keywords: Oxidative stress, Nonsyndromic cleft lip with/without cleft palate, MDA

P-151
Synergistic antioxidant effects of melatonin and arginine at the cerebral and hepatic level

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OBJECTIVES:Biological structures are susceptible to oxidative stress and lipid peroxidation having a limited number of bio molecules active as antioxidants at physiological concentrations. The objective of the study was to determine the antioxidant capacity of melatonin, the main pineal hormone with multiple biological roles as antioxidant, anti-aging, DNA defense agent and neuroprotector, and arginine, a semi-essential amino acid studied for its effects in cell division, immune system modulation and carcinogenesis, and as a precursor of NO synthesis.
MATERIALS and METHODS:White male Albino Swiss mice were randomized in 4 groups administered for a period of three weeks with arginine and melatonin, single or in combination. The hepatic and brain tissue homogenates were subjected to the assessement of lipid peroxides, in temporal dynamics, by the reaction with thiobarbituric acid, the results being expressed in nmol malondialdehyde/mg protein.
RESULTS:At the cerebral level, the dynamics of the lipid peroxidation process recorded interesting values demonstrating protective effects for the studied substances against oxidative processes, the best results being obtained for arginine + melatonin treated groups. In the hepatic tissue, melatonin registered the lowest antioxidant effect, but it was achieved a substantial improvement by its association with arginine.
CONCLUSIONS:The lipid peroxidation process is a characteristic of each tissue, depending on the intensity of cellular oxidative processes and the mechanisms underlying the balance between pro-oxidant factors and antioxidants. The obtained results demonstrate the synergistic effect of melatonin and arginine as effective modulators of redox processes, significantly diminishing the tissue potential of lipid peroxidation.
Keywords: arginine, melatonin, lipid peroxidation, malondialdehyde, oxidative stress

P-152
Superoxide dismutase – first line defence antioxidant enzyme in women with polycystic ovary syndrome

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OBJECTIVES:Oxidative stress is a condition which occurs as a result of physiological imbalance between the levels of antioxidants and oxidants (ROS - reactive oxygen species) in favour of oxidants. It causes oxidative damage and processes that happen in the body contribute to the development of a number of interrelated risk factors as hyperglycemia, dyslipidemia, hyperinsulinemia, insulin resistance. The effects of an increased oxidative load are reduced by antioxidant enzymes that convert ROS to less harmful molecules. Superoxide dismutase (SOD) is the first detoxification enzyme and most powerful antioxidant in the cell. The aim of our study is to evaluate changes in serum SOD in women with polycystic ovary syndrome (PCOS).
MATERIALS and METHODS:The study includes 55 women, divided into two groups: 29 women with PCOS and 26 clinically healthy women. Serum SOD was determined with an ELISA kit (MyBioSource, USA). SOD concentrations were measured by a multiparameter photometer "Sirio S microplate reader", SEAC, Italy. All data was presented as mean ± SD. Significance was defined as $P < 0.05$.
RESULTS:The mean age of women with PCOS was 25.03 ± 4.94 yrs. and of healthy women was 30.34 ± 5.76 yrs. Serum concentrations of SOD were significantly lower in women with PCOS compared with healthy controls (14,06 ± 3,17 vs 38.95 ± 45.32, $P < 0.001$).
CONCLUSIONS:Our results indicate decreased SOD serum concentrations in studied women. It is believed that serum SOD could be a helpful biomarker in assessment of oxidative stress in women with PCOS.
Keywords: SOD, PCOS, oxidative stress

P-154
Effect of tourniquet usage on ADMA levels undergoing unilateral total knee arthroplasty patients

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OBJECTIVES:Pneumatic tourniquets are commonly used in the orthopedic field to reduce blood loss and maintain a clear surgical field in limb surgery. Despite their beneficial effects, tourniquet-related adverse effects such as vascular injury and limb ischemia–reperfusion injury have been identified in many studies. The aim of our study was to investigate the effect of tourniquet usage on preoperative and postoperative (1th and 24th hours) ADMA, SDMA, L-NMMA, arginine, citrulline levels undergoing unilateral total knee arthroplasty (UTKA) patients.
MATERIALS and METHODS:31 patients who underwent UTKA with or without tourniquet in Selcuk University Faculty of Medicine Clinic of Orthopedics and Traumatology were included in the study. All parameters were analyzed by LC unit coupled to an ABSCIEX API 3200 mass spectrometer. Paired sample t test was used for statistical analysis. $p <0.05$ was taken to be statistically significant.
RESULTS:It was determined a reduction between ADMA0-ADMA24, Cit0-Cit1, Cit0-Cit24 (with tourniquet), Cit0-Cit24 (without tourniquet) periods ($p=0.002$; $p=0.025$; $p=0.001$; $p=0.003$ respectively). There were no significant differences in other periods and parameters, both two operation methods.
CONCLUSIONS:In many studies, ıt was reported that usage of tourniquet during surgery increases oxidative stress status depending on ischemia in knee arthroplasty. Oxidative stress has been shown to increase the activity of arginine methylating and ADMA degrading enzymes leading to increased ADMA concentrations. When the results obtained in our study were evaluated, it was observed that in the long term ADMA levels decreased, in the short and long term citrulline levels decreased in the patients who were operated with tourniquet.
Keywords: Knee arthroplasty, tourniquet usage, oxidative stress, ADMA

P-155
Oxidative status in patients with inflammatory bowel disease

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OBJECTIVES: To evaluate the role of free radical oxidation and antioxidant defense for the progression and activation of inflammatory bowel disease (IBD).
MATERIALS and METHODS: 54 IBD patients (mean age 44,5±14,3y) and 80 healthy age-matched controls (43±10.8y) were enrolled in the study. According to CDAI and Mayo indexes, the patients were divided into two subgroups: moderate/severe activity (36 patients) and remission/mild activity (18 patients). CRP and fecal calprotectin were measured as inflammatory markers. Hydroperoxide levels and serum antioxidant capacity were evaluated using commercial kits dROMs and BAP-test (Diacron Labs, Italy). Standard statistical methods (descriptive statistics, Student's t-test, and Spearmann correlation) were used for data analysis.
RESULTS: Significantly increased levels of dROMs were measured in IBD patients vs controls (418.1±124.4UCarr vs 341.2±37.48UCarr, p<0.0001). Patients with active form of IBD revealed significantly higher dROMs compared to mild/remission subgroup (437.8±131.4UCarr vs 357.0±81.74UCarr, p<0.05). Serum antioxidant capacity was significantly decreased in the IBD group vs controls (2122±468.6umol/l vs 2683±279.9umol/l, p<0.0001). A tendency for weaken antioxidant defense was found with the severity of the disease (2047±608.9umol/l for the subgroup with moderate/severe activity and 2206±432.8umol/l for the mild/remission subgroup). The increase of dROMs was significantly associated with CRP (Spearman r=0.5545, p<0.0001) and calprotectin levels (Spearman r=0.3295, p<0.05). BAP-test correlated negatively with CRP levels (Spearman r=-0.5419, p<0.0001) and with calprotectin (Spearman r=-0.2078, ns).
CONCLUSIONS: Increased free radical oxidation and diminished antioxidant defense with the severity of the disease and their associations with routine inflammatory markers suggest a possible role of oxidative stress in the pathogenesis of IBD.
Keywords: IBD, oxidative stress, antioxidant defense, CRP, calprotectin

P-156
Nitric oxide increased in preeclampsy independently from malondialdehyde

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OBJECTIVES: Preeclampsia, which is a complication that usually develops in the later stages of pregnancy, is still being debated in relation to nitric oxide (NO) and oxidant / antioxidant system. The aim of this study was to determine the maternal serum concentrations of nitric oxide and malondialdehyde (MDA) in preeclamptic pregnancies and to compare them with healthy patients.
MATERIALS and METHODS: The study included 38 pregnant women with a gestational age of 30-38 weeks with preeclampsia and 42 normotensive pregnant women with the same gestational week. Serum NO levels were measured by the Griess method as described by the colorimetric assay kit manufacturer (NB98, Oxford Biomedical Research). The absorbance of a pink complex formed after MDA reacted with thiobarbituric acid was measured spectrophotometrically at 535 nm.
RESULTS: In our study, serum NO levels of preeclampsia pregnant women were higher than healthy pregnant women (P <0.05). However, there was no difference between the groups in terms of MDA levels (P> 0.05). In addition, there was no statistically significant correlation between NO and MDA levels of all participants (Spearman r = -0.01169 P = 0.9180).
CONCLUSIONS: Especially in preeclampsia patients who developed after 30th week, NO levels were found to increased. However, this increase was not associated with MDA, an indicator of oxidative stress.
Keywords: Preeclampsia, nitric oxide, malondialdehyde

P-157
Haptoglobin polymorphism may cause atherosclerotic changes

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OBJECTIVES:Human gene of haptoglobin is presented by two alleles. Haptoglobin types are 1-1, 1-2 and 2-2. Different studies shows role of type 2-2 in cardio-vascular disease occurrence during diabetes. Haptoglobin type 1 is known to suppress hemoglobin based oxygenation of HDL and LDL, acting like antioxidant.
MATERIALS and METHODS:We aimed that Bulgarian population is haptoglobin 2-2 type, which causes frequent morbidity by systematic diseases, such as atherosclerosis, diabetes, diabetic nephropathies, gestational diabetes, anemia, etc. 39 volunteers were included, age 36.7 ± 5.3. IMT, ABI, CBC, iron homeostasis, hsCRP and haptoglobin type were evaluated.
RESULTS:Increased serum hepcidin concentrations were established in patients with atherosclerotic a. carotis changes (109.7 ± 10.1 µg/L) compared to healthy controls (21.1 ± 1.9 µg/L), P<0.001. In haptoglobin type 2-2, was found strong positive correlation between hepcidin levels and changed IMT and ABI (r=0.901, r=0.919, resp.; P<0.01). Three volunteers were with haptoglobin type 2-1; no changes of serum hepcidin concentration and IMT, ABI was found in this phenotype.
CONCLUSIONS:The main reason for acute coronary thrombosis is atherosclerotic plaque rupture. Extra-vascular hemoglobin plays role as start mechanism for inflammation in the plaques. Important contra-active mechanism is played by haptoglobin. Thus, it prevents kidney injury from free hemoglobin. Released iron from destructed erythrocytes forms reactive oxygen radicals through Fenton's reaction. Hepcidin regulates iron homeostasis by its interaction with intracellular iron exporter ferroportin.
Acknowledgements: This project is sponsored by MU-Sofia, as part of Grant Д-213/2018.
Keywords: oxidative stress, haptoglobin, atherosclerosis, iron

P-158
Determination of glucose levels in sports active children

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OBJECTIVES:Physical activity is a very important part of healthy lifestyle in children and adolescents. Physical activity is known to increase glucose consumption, which is used as an energy source, thereby reducing blood glucose levels.
MATERIALS and METHODS:Sports active children included children who were actively involved in sports in addition to physical activity at school. It included 158 healthy children (82 boys and 76 girls) aged 4-6 years and 12-14 years. There were 76 children active in sports and 82 inactive. Glucose levels have been determined by routine laboratory methods.
RESULTS:Comparisons are made by age and gender in relation to whether or not they are involved in sports. In the group of younger children and older boys there is no statistically significant difference in glucose level.Comparing glucose level in girls group, glucose levels were significantly lower (p <0.01) in girls engaged in sports activity versus girls inactive in sport (4.95 ± 0.2 mmol/L versus 5.20 ± 0.32 mmol/L).
CONCLUSIONS:Based on the results, we can conclude that although there was no difference in glucose concentration in young children, the puberty period is critical and special attention should be paid to prevention. Playing sports has the effect of improving health and quality of life. Diet and

exercise should be adapted to each child. Systematic examinations of children involved in sports should be regular and should include control of the risk parameter for the development of diabetes mellitus as well as the parameters for the development of cardiovascular disease.
Keywords: Children. Sports. Glucose

P-161
Screening for hypothyroidism

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OBJECTIVES:Hypothyroidism is a clinical condition that arises out of the development defects of the thyroid gland.The most frequently endocrinologic problem encountered reason of permanent hypothyroidism is the congenital reasons.Incidences in our country, hypothyroidism was found as one per every 2183 alive births.Tests are conducted using the National Newborn Screening Program developed Ministry of Health since 2006.
MATERIALS and METHODS:The TSH eliza method is employed for hypothyroidism at a threshold value of up to 15 mg/dl. Since the symptoms and findings of early diagnosis sometimes is difficult. In non-treated cases, serious mental retardation but treatment is easy, inexpensive, and efficient. In Avcılar Hospital, number of alive births for the year 2017 is 721. Heel blood is taken from all newborns after at least 24 hours of feeding, and a Guthrie card is filled in.If the blood samples taken here are insufficient, repetition is requested.
RESULTS:Appropriate samples are worked on in the screening laboratory, and any results above the normal values are taken into further examination. Screening test results examined in the hospital for the congenital hyperthyroidism are as follows: TSH was above normal level in 11. According to these results, 4% samples in total among the babies born in this hospital were required to be taken again. Among these, 0.3% were required to be redirected to the concerning clinic for further follow up.
CONCLUSIONS:Sampling from all alive births in the hospitals and efficient participation of big centers such as the maternity wards into screening purpose surveys makes contribution to obtaining the country-wise data.
Keywords: Hypothyroidism

P-162
Evaluation of pediatric coagulation tubes

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OBJECTIVES: In this project,we aimed to evaluate the performance of pediatric tubes by comparing the results of 2.7 ml coagulation tubes containing %3.2 sodium citrate and 0.5 ml pediatric tubes containing %3.2 sodium citrate.
MATERIALS and METHODS: In addition to the standard coagulation sample,0.5 mı of blood was collected from our adult volunteers over 18 years old who applied to our hospital for coagulation tests. Prothrombin Time(PT), Activated Partial Thromboplastin time(aPTT) and Fibrinogen tests were analyzed from these two samples on Sta Compact Max. We then compared the results statistically.
RESULTS: No statistically significant results were found between PT, aPTT, fibrinogen test results of pediatric coagulation tubes and normal coagulation tubes..
CONCLUSIONS: Pediatric coagulation tubes may be used in pediatric patients or in adult patients with difficult blood collection.
Keywords: Pediatric coagulation tubes,PT,aPTT, Fibrinojen,coagulation tubes

P-163
A method comparison study of a novel point of care test for hemolysis detection in vacuum tubes

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OBJECTIVES:Hemolysis is a frequent pre-analytical error accounting for up to 70% of all rejected specimen in clinical laboratorys. Considering that biochemical analysis impact clinical outcomes, errors may subject patients to adverse effects and place an unnecessary economic burden on a hospital budget. A line of evidence indicates that hemolysis usually is traced to the blood collection. To mitigate this an effective approach could be to identify hemolysis at the point of care. Here a novel method for point-of-care hemolysis detection is evaluated.
MATERIALS and METHODS:Lithium heparin tubes part of routine care was collected and roughly 100 µL whole blood was analyzed for hemolysis in plasma with Helge (Helge, Hemcheck, Karlstad, Sweden) at an emergency department (ED) in Sweden. Results were recorded at the ED, and samples were sent with pneumatic dispatch to central laboratory for routine handling. Hemolysis index was collected from the reference method Vitros 5.1 FS (Ortho Diagnostics Inc. New Jersey, United States). Clinically relevant hemolysis was 0.5 g/L free hemoglobin.
RESULTS:794 samples were collected during four weeks for calculation of performance. The proportion of hemolytic samples was 9.9% (n=79) according to the reference method. The sensitivity and specificity of Helge were 81.0% and 97.8% respectively. The positive and negative predictive values were 80.0% and 97.9% respectively.
CONCLUSIONS:Hemolysis is a frequent pre-analytical error, in this study 9.9% of included blood samples were rejected. If a non hemolyzed sample could be taken following a positive test, in this study, the proportion of rejected samples would be reduced from 9.9% to 1.9%.
Keywords: Hemolysis Point-of-Care Systems Pre-Analytical Phase

P-164
The effect of storage conditions on prenatal screening tests

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OBJECTIVES:Prenatal screening tends to be performed in central hospitals as it requires expertise for interpretation of results and quality management and cost effectiveness as well. For this reason, samples taken from peripheral hospitals are transferred to the central laboratory on a certain day of the week. During this period, samples can be frozen and stored. The aim of this study is to investigate the effect of storage on prenatal screening tests and risk analysis.
MATERIALS and METHODS:TotalβhCG,PAPP-A(n17) for double screening; total βhCG,AFP,uE3,inhibin-A(n:21) for quadruple screening were studied on freshly drawn blood samples on Beckman-Coulter-Access2 analyzer on the same day. Risk assessment was performed in BenetechPRA software. The cutoff value was 1/250 for the risk of Down syndrome. The same serum samples were frozen immediately and thawed after one week of freezing at -30°C and the tests were re-studied to calculate the risk analysis.
RESULTS:The median and IQR(Q1-Q3) values of fresh and frozen samples were as following: PAPP-A:617(417-1195)µg/L and 671(472-1252)µg/L;totalβhCG:117030(75836-174448)IU/L and 100007(77363-196865)IU/L in double screening; and AFP:31.6(21.6-42.4) ng/mL and 37.0(25.0-44.5) ng/mL;total βhCG:57415(40687-83812)IU/L and 62800(47078-93462)IU/L;inhibin-A:284(204-458)pg/mL and 315(224-471)pg/mL in quadruple screening, respectively. The difference was statistically significant between fresh and frozen samples($p<0.05$). There wasnt a statistical difference between uE3 results. Risk analysis also did not showed a difference after storage($p>0.05$).
CONCLUSIONS:Freezing and thawing of the samples did not change the risk analysis, although the test results could change. In addition to biochemical tests, maternal characteristics such as maternal age, weight, race, diabetes mellitus, smoking and USG findings are effective in calculating the risk of Down syndrome.
Keywords: Prenatal screening tests, risk analysis, fresh sample, frozen sample

P-165
The effect of hormones secreted by skin contact on the separation time of the placenta

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OBJECTIVES:This study was aimed to investigate the effect of hormones on the duration of separation of plasma skin to skin contact.
MATERIALS and METHODS:The study was conducted with 20 cases, 20 controls. Blood samples were taken during routine check-up before and after the birth of 1 cc of blood for our study. Blood samples were stored in the deep freezer at -80 degrees until all of the bloods were collected. Then, beta-endorphins, catecholamines and oxytocin were analyzed. Data were taken using the socio-demographic data form. In addition, the effect of skin to skin contact on placenta seperation time was measured with an observational chronometer. The Mann-Whitney U test was used to evaluate the data.
RESULTS:The mean age of mothers in the case group was 28,55±5,97, the mean age of mothers in the control group was 26,75±6,58. Statistically, the levels of oxytocin in control prepartum and case prepartum groups decreased, while beta-endorphin levels increased and catecholamine levels did not change. There is no significant difference between control postpartum and case postpartum groups in terms of oxytocin, beta-endorphin and catecholamine levels (p>0,05). In addition, the seperation time of the placenta was shorter in the case group compared to the control group. There is a statistically significant difference between them (p<0,05).
CONCLUSIONS:Skin to skin contact at birth is a factor affecting the separation time of the placenta. Health professionals should be informed and awareness about skin to skin contact should be increased in the early postpartum period.
Keywords: Skin to skin contact, oxytocin, beta-endorphin, catecholamine, placenta

P-166
Determination of median values of biochemical parameters in double and quadruple prenatal screening tests

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OBJECTIVES: In this study, we aimed to determine the median values of the biochemical parameters of the prenatal screening tests of double (DS) (PAPP-A and total ß-hCG) and quadruple (QS) (AFP, uE3, total ß-hCG, Inhibin A) and to compare with the median values given by the company.
MATERIALS and METHODS: Data of 2111 pregnant women between 11-13 weeks for DS and 1683 pregnant women between 15-19 weeks for QS were included in the study.Pregnants with diabetes, IVF and twin pregnancy, smoking and were excluded. Pregnant women with a risk of Smith-Lemli-Opitz syndrome and above the threshold of 1/250 for Down syndrome, 1/300 for trisomy 18, 1/104 for neural tube defect were also excluded.All analyses were performed on a Beckman-Coulter Access2 analyzer and risk analysis was performed with Benetech PRA.The "sign test for medians" was used for the comparison of medians.
RESULTS: For DS, the new median values were significantly different(p <0.05) for all three weeks(11-13.) than the medians recommended by the company.For QS, the median of inhibin A at weeks 15th-19th, AFP at weeks 15-17th, uE3 at weeks 15-17th and 19th, total ß-hCG was significantly different at the only 17th week(p <0.05) compared to the default medians.
CONCLUSIONS: Median values of biochemical parameters in prenatal screening tests may vary according to geographical regions.Newly found medians are different from than the default ones.This may be due to the large number of refugees coming to our country in recent years.Thus, in terms of maternal and fetal safety, each laboratory should calculate and use its own median values.
Keywords: double prenatal screening test, quadruple prenatal screening test, median value

P-167
Relationship between maternal TSH and first trimester screening parameters

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OBJECTIVES:Between 11-14th weeks (1st trimester) ß-hCG and PAPP-A are examined from maternal serum to determine the risk of trisomy 21.Higher hCG values increase the risk of trisomy 21.HCG and TSH hormones consist of alpha and beta subunits and alpha subunits are structurally similar.High levels of HCG during pregnancy show thyrotropic effect by binding to TSH receptors.The aim of this study is to investigate the relationship between TSH and ß-hCG levels during the first trimester.
MATERIALS and METHODS:The study included 331 pregnant women investigated for first trimester scrreening and had TSH request simultaneously. Of these 52 pregnants had also simultaneous free T4 requests.Total β-hCG and PAPP-A were studied on a Beckman-Coulter Access2 and TSH on a Roche Cobas 6000 e601 analyzer.Risk analysis was performed in Benetech PRA software.The study group was divided in three subgroups according to TSH values:<0.1mIU/L,0.1-2.5mIU/L and >2.5mIU/L as hyperthyroid,euthyroid and hypothyroid, respectively.The relationship between the analytes in these three subgroups was analysed and risk analysis was re-evaluated.
RESULTS:There was a negatively significant weak correlation between TSH and total β-hCG(r =-0.125; p<0.05) and TSH and Down syndrome risk(r=-0.147; p <0.01).There was no significant relationship between TSH and PAPP-A.There was a statistically significant difference between TSH subgroups for total β-hCG MoM(p=0.003).There was no significant relationship between TSH subgroups and total β-HCG.There was a nonsignificant weak correlation between T4 and TSH(r =-0.217; p>0.1), and free T4 with total β-HCG(r=0.203, p>0.1).
CONCLUSIONS:In the first trimester,increased hCG may affect the thyroid function.Therefore,thyroid disease and drug use should also be taken into consideration during pregnancy.
Keywords: TSH, maternal screening, down syndrome, correlation

P-168
Evaluation of the effect of measurement uncertainty on risk analysis in prenatal screening

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OBJECTIVES: Increased ß-hCG and inhibin-A values, decreased AFP and uE3 values increase risk of Down syndrome (DS). Higher AFP concentrations increase risk of NTD. The aim of this study is to calculate measurement uncertainty (MU) of analytes used in quadruple screening and to perform risk analysis again considering worst probability.
MATERIALS and METHODS: MU of each parameter was calculated according to Nordtest NTTR537 and ISO/TS21748 guidances. Analytes were studied on Beckman-Coulter Access2 analyzer and risk analysis was performed on Benetech PRA software. 200 consecutive patients were included; patients were divided into two groups as 35 years and older; and younger (n=20 and n=180, respectively). Cut-off values for DS and NTD were taken as 1/250 and 1/104, respectively. First risk analysis was compared with the worst probability risk analysis considering MU.
RESULTS: Extended MUs% of total ß-hCG, AFP, uE3 and inhibin-A analytes were ± 25.46, ± 21.82, ± 11.17 and ± 25.25, respectively. When all patients (n=200) were considered, 8 patients had a positive risk for DS and this number increased to 40. Risk for NTD increased from 2 to 5. In patients <35 years, risk for DS increased from 8 to 34, while the number for NTD did not change.
CONCLUSIONS: It was found that pregnant women with low risk were not affected but clinically significant risk increase was found in pregnant women with close to cut-off. MU can be given with test result in results close to cut-off value in prenatal screening tests. It would be useful to inform clinicians on this issue.
Keywords: Prenatal screening test, Measurement uncertainty, Risk estimation

P-169
Causes of sample rejection in medical biochemistry laboratory of Gaziantep University Research and Practice Hospital

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OBJECTIVES:The management of preanalytical errors is important for efficient and reliable analyze of patient results. The objective of this study is to determine, classify and evaluate the frequency of sample rejections in the preanalytical phase.
MATERIALS and METHODS:The samples sent to our Laboratory between of January 1st 2019 and June 30th 2019 were analyzed.The data obtained were proportioned and all the rejection reasons were evaluated according to the frequency rates.
RESULTS:Between the dates mentioned above, our Laboratory received 950.554 patient sample. 17.518 samples were rejected. Thus, 1,84% of all samples were rejected.The rejected samples were received from adult emergency(18.87%), intensive care units(11.59%), pediatric emergency(6.85%) and other services and clinics(62.69%).When all the results were analyzed, it has been found that the most frequent rejection rates were in neonatal intensive care unit(10,59%), pediatric cardiology(8.84%), infectious diseases service(8,69%) and child health and diseases department(7,31%).Comparing the rejection rates among the intensive care units, the highest rates were found in neonatal intensive care unit(10,59%), pediatric intensive care unit(4,92%) and thoracic surgery intensive care unit(4,86%).The most frequent causes of sample rejections within the test groups are found as insufficient sample(33,69%), hemolyzed sample(19,11%) and clotted sample(17,77%).Other rejection causes were found as taking samples at the wrong level, inappropriate test requests and samples which are not delivered to the laboratory.
CONCLUSIONS:Detecting and documenting the problems are important. Evaluating the results, a training program named “Bloodletting and Sample Transfer” was held in June. We aim to decrease the number of defined error rates through increasing the frequency of training programs.
Keywords: Preanalytical errors, sample rejection

P-171
Evaluation of analytical process performance of ethanol with Six Sigma values

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OBJECTIVES:Today, within the scope of the performance evaluation of analytical quality, internal quality control (IQC) and external quality control (EQC) applications are made in clinical laboratories. On the other hand Sigma metrics have become a useful tool for all parts of the quality control (QC) design process. We aimed to evaluate the Six Sigma Methodology for Analytical Process Performance Assessment using ethanol test results in our study
MATERIALS and METHODS:The Analytical Process Performance was evaluated according to the Six Sigma methodology by taking advantage of the IQC and EQC results for April, May, June of 2019 for ethanol. Coefficient of variance (CV) was calculated from IQC for two level quality control material. Percentage bias for these parameters was calculated from the RIQAS. Total allowable errors were followed as per Clinical Laboratory Improvement Amendments (CLIA) guidelines.
RESULTS:QC-2 sigma values were found to be more than 6, but QC-1 sigma values were found be between 4 to 5.
CONCLUSIONS:Ethanol results for QC -1 level signifying more QC rules to be implemented. No significant difference was found in context to sigma value in April, May, June of 2019 ethanol results. Six Sigma Methodology allows laboratories to easily visualize performance, optimize the QC rules and numbers of control measurements.
Keywords: Six Sigma Methodology, Ethanol, Total allowable error, Bias, Coefficient of variance

P-172
Usability of exponentially weighted moving average on patient based quality control

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OBJECTIVES:Conventional quality control (QC) approach requires periodic analysis of QC samples within predetermined frequency which can be as few as once per day. As many systematic errors may be overlooked with this approach, patient-based QC monitoring real-time patient results has attracted attention. We aimed to investigate the usability of exponentially weighted moving average (EWMA) on patient-based QC via a simulation study.
MATERIALS and METHODS:Patient results within reference intervals representing normal distribution were generated for 10 analytes including sodium, potassium, calcium, urea, creatinine, AST, CRP, free thyroxine (fT4), thyroid stimulating hormone (TSH) and prolactin. 1,000,000 results (n=500 per day, d=2,000) were produced. For each day, four gradually increasing systematic errors (SE) were added seperately starting from every 100th result. The maximum value of %SE added to the results was adjusted to correspond total allowable error(TEa). The average number of patient samples affected until error detection(ANPed) and optimum weighting factors for stated analytes were determined. ANPed-%SE graph was plotted to calculate the area under the curve (AUC) and reveal optimum weighting factors.
RESULTS:Optimum weighing factors and corresponding minimum AUC values are 0.1;16.1, 0.1;38.7, 0.1;12.7, 0.4;3.7, 0.1;9.17, 0.1;10.5, 0.1;28.8, 0.2;1.21, 0.1;36.3 and 0.1;23.9 for AST, CRP, fT4, calcium, creatinine, potassium, prolactin, sodium, TSH and urea, respectively. Weighting factors greater than or equal to 0.5, 0.7, 0.8 and 0.5 were found to be unable to detect any %SE up to TEa for calcium, TSH, urea and potassium, respectively.
CONCLUSIONS:Outcomes of present study elucidated both optimum and useless weighting factors of EWMA for 10 common analytes.
Keywords: Exponentially Weighted Moving Average, Patient Based Quality Control, Quality Control, Systematic Error, Quality Management

P-173
Assessment of critical values notification in a Turkish clinical biochemistry laboratory

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OBJECTIVES:The critical values are the laboratory testing results which required attention or action by the physicians. The aim of this study was to investigate critical value results from our laboratory and compare our critical value prevalence with others in the literature
MATERIALS and METHODS:The study was conducted by retrospectively in the Balıkesir State Hospital. In this retrospective study, We performed analysis of critical values from data obtained by the laboratory information system during 2 years. The critical results were identified by clinical chemistry laboratory according to guidelines.
RESULTS:The critical values was found by 0.5% of total laboratory tests. We determined 4736 critical values notification, of which 20.4% came from emergency units, 44.9% from intensive care units, 15.3% from routine inpatients and 19.4% from routine outpatients. The highest rate of critical values was shown for oxygen partial pressure(pO2) (21.1%), followed by white blood cell(WBC) and platelet (PLT)(11.7% and 10.9%) concentrations. According to department, the highest rate of the critical value notification were pO2, glucose, WBC and potassium ion concentrations for emergency patients, were PLT, WBC, and hemoglobin phosphate concentrations for inpatients and, were WBC, pO2 and prothrombin time concentrations for outpatients. Mean time for notification for all departments was 12 min.
CONCLUSIONS:The analysis of critical values notification in our hospital is

in suitable with that declared in the literature. This study will contribute in the establishment of international harmonized postanalytical phase-related criteria and indicators of the critical values notification
Keywords: Critical values, critical values notification, post-analytical phase, patient safety

P-174
Monitoring of quality indicators in preanalytical phase of laboratory testing process

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OBJECTIVES:Quality indicators (QIs) are fundamental tools enabling users to quantify the quality of laboratory services. Preanalytical variables account for 32-75% of laboratory errors and encompass the time from when the test is ordered by the physician until the sample is ready for analysis. Aim of this study is to quantify performance in the pre-analytical phase of testing in Medical Biochemistry Laboratory of SBÜ Ankara SUAM using quality indicators and to assess the quality of our laboratory services.
MATERIALS and METHODS:Pre-analytical process error data between 1 st July 2017 – 31 st June 2019 were obtained from the laboratory information management system. Every type of error percentages have been calculated and evaluated according to the Quality Indicators developed by the IFCC Working Group on "Laboratory Errors and Patient Safety" (WG-LEPS).
RESULTS:A total of 2 496 748 samples received to our laboratory. 33 939 of them were rejected, giving a rejection rate of 1.4 %. The main causes of sample rejection were clot formation (38.3%) and hemolysis (32.3%). The other sample rejection reasons were inadequate sample volume (24.4 %), incorrect samples (7.8%) and missing tests (4.5%). When these results were compared with specifications of IFCC (WG-LEPS): QI-7, QI-9, QI-10 and QI-12 were found to be within optimal level whereas QI-11 was within desirable range. Sigma values also were within acceptable range.
CONCLUSIONS:The preanalytical performance of our laboratory is favorable and complies with international quality specifications.
Keywords: Preanalytical error, quality indicator, sigma metric

P-176
Analytical process evaluation of biochemistry laboratory of Patnos State Hospital

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OBJECTIVES:The primary purpose of medical laboratories is to provide the most accurate and reliable results appropriate to the patient's medical condition. Therefore, the reliability of each laboratory must be scientifically proven. Approximately 15% of laboratory errors occur in the analytical phase. To evaluate the analytical process of laboratory, we aimed to perform performance evaluation according to six sigma methodology. The tests evaluated for this purpose; albumin(Alb), alanine aminotransferase(ALT), aspartate aminotransferase(AST), chlorid(Cl), total cholesterol(TChol), creatinine(Crea), glucose(Glu), HDL cholesterol(HDL-C), lactate dehydrogenase(LD), potassium(K), total protein(TP), sodium(Na), triglyceride(Tg) and blood-urea-nitrogen(BUN).
MATERIALS and METHODS:Mean, standard deviation(SD) and coefficients of variation(%CV) were calculated from the 1-month internal quality control data of the 14 most frequently used biochemistry parameters in the laboratory (Roche-Cobas-c501). Bias was determined using the control target value of the firm. Acceptable total error(%Tea), was determined according to the CLIA and Turkey(TR) criteria. Sigma values were calculated via (%TEa -% Bias)/%CV formula. According to sigma levels; <3 unacceptable; 3–6 are acceptable; ≥6 world-class performance, divided into three groups.
RESULTS:According to the CLIA sigma assessment, both levels of Cl and Na and the second level of Alb's performance were unacceptable, other tests were found to be acceptable or world-class performance while according to TR sigma assessment, all tests were acceptable or world-class performance.
CONCLUSIONS:Sigma measurements should be routinely performed in laboratories to assess the analytical period performance of the laboratory and improve its quality through regulatory preventive actions. Our study allowed us to see and improve our measurement quality by determining the 1-month-periodic performance of laboratory tests.
Keywords: Internal quality control, Analytical performance assessment, Six sigma methodology, Total allowable error (Tea)

P-177
Assessment of vitamin D levels using hospital data in a Turkish clinical biochemistry laboratory

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OBJECTIVES:Vitamin D deficiency is a public health concern worldwide and defined as a 25(OH) vitamin D3 (25(OH)D) level less than 12 ng/mL. This study aimed to evaluate the 25(OH)D levels in the clinical laboratory of Bursa Uludag University and estimate vitamin D deficiency in adults.
MATERIALS and METHODS:The results of 25(OH)D levels from 44873 outpatients (13417 males, 31417 females) aged 18-65 years were collected from the laboratory information system for a period of 3 years. Chemiluminescent Microparticle Immunoassay on Architect i2000SR analyzer of Abbott was used for the measurement of 25(OH)D levels. The Architect 25(OH)D assay demonstrated linearity from 3.4 to 155.9 ng/mL.
RESULTS:Vitamin D levels lower than 12 ng/mL were observed in 17532 of 44873 patients in total (39.0%), 3641 of 13417 males (27.1%) and 13891 of 31417 females (44.1%). The median values of all subjects, males and females were 14.4, 17.1 and 13.2 ng/mL, respectively. The mean (+/- SD) vitamin D levels of all subjects was 17.48 +/- 14.1 ng/mL with the value for females being lower at 16.77 +/- 14.7 ng/mL com¬pared to males at 19.10 +/- 12.2 ng/mL and the difference was statistically significant (p<0.001).
CONCLUSIONS:The prevalence of low vitamin D levels may be increasing globally. Data from the NHANES in the US showed a decrease in mean 25(OH) D concentrations from 24 to 19.9 ng/mL. However, our data shows that in Turks 25(OH)D concentrations are lower than these values and vitamin D deficiency may be more prominent in Turkey.
Keywords: 25(OH) vitamin D3, Vitamin D deficiency, Turkish adults, Laboratory data

P-178
Determination of whole blood reference ıntervals from hospital data – A Bhattacharya analysis

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OBJECTIVES:Determination of reference intervals (RIs) by direct method is difficult and expensive. Therefore, many laboratories use the RIs recommended by the manufacturer. This may cause problems due to ethnic, genetic and environmental differences. In our study, we aimed to determine the RIs of the parameters measured in blood cell count (BCC) by using the patient data recorded in the Hospital Information Management System (HIMS) and to compare them with those used in our laboratory.
MATERIALS and METHODS:Between January-December 2017, 101071 patients who applied to outpatient clinics of eye, ear nose and throat, physical therapy rehabilitation, urology, orthopedics, general surgery, plastic and reconstructive surgery, intact children, internal medicine and health board were included in the study. BCC parameters were analysed by Sysmex XN-3000 and XN-2000 instruments. The RIs were calculated separately for the female and male sexes for the 11-14, 15-20, 21-29, 30-39, 40-49, 50-64 and ≥65 age groups

and without discrimination of gender in the 1-10 age group by using indirect Bhattacharya method. IBM SPSS Statistics 22 program was used to exclude outliers and macros prepared in Microsoft Excel was used in calculations.
RESULTS:Most of the calculated RIs were consistent with the RIs that currently used in our laboratory. However, there were differences in the RIs of hemoglobin, RBC, MCH, MCHC, RDV-CV, PLT and HCT in different age and sex groups.
CONCLUSIONS:RIs can be determined by indirect method according to IFCC and CLSI recommendations from big data stored in HIMS. This approach may add value on patient safety.
Keywords: reference intervals, big data, bhattacharya analysis, blood cell count

P-180
Use of big data for verification of decision levels for biotinidase deficiency and galactosemia

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OBJECTIVES:To decide for further testing, the percentages of mean/median enzyme activity of individuals, and cutoff are needed for biotinidase (BTD) deficiency and galactosemia (GALT), respectively. Each laboratory that performs screening tests should estimate these decision levels. The mean (263 Enzyme Unit-Eu) and the cut-off (3.5 U/g Hb) were determined for BTD and GALT, respectively. The reference intervals (RIs) are being estimated from big data. Our aim is to assess whether these values determined from small numbers of healthy newborns will be similar with the values estimated by the indirect methods for determination of RIs.
MATERIALS and METHODS:The histograms and Q-Q Plots of 33998 BTD, and 23438 GALT screening results generated in Tanyalçın Laboratory from 2004 were evaluated. The RIs were estimated according to the Hoffmann and Bhattacharya Methods. The Microsoft Excel and SPSS Statistical Package were used.
RESULTS:Hoffmann METHOD: The data was separated into two sets according to the Q-Q Plots. The outliers were removed using the Tukey's Method. The mean (SD), median, 2.5-97.5 percentiles are estimated as 247(81), 254, and 80-384 Eu (N=33226) for BTD; and 8.29(2.21), 8.36, 3.75-12.79 U/g Hb (N=21309) for GALT, respectively. Bhattacharya Method: The center (SD), lower-upper limits were found as 270(84) and 102-438 Eu (N=33 364) for BTD (h=30), and 9.5(3.09), 3.30-15.69 U/g Hb (N=22862) for GALT (h=2), respectively. The values determined were compatible with the decision levels estimated before.
CONCLUSIONS:The indirect methods for RI determination from big data can be helpful for verification of decision levels for the screening tests.
Keywords: Indirect methods, reference interval, big data, biotinidase deficiency, galactosemia

P-181
Reference values of neutrophil-lymphocyte, lymphocyte-monoctye, platelet-lymphocyte ratio and mean platelet volume in healthy adults

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OBJECTIVES:This study was designed to evaluate the gender and age-specific reference values of neutrophil-lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMR), platelet-lymphocyte ratio (PLR) and mean platelet volume (MPV) which are indicative of systemic inflammation.
MATERIALS and METHODS:The results of the patients admitted to the outpatient clinics of our hospital were collected between January 2017 and July 2019. Total number of patients was 249 829; 100 195 (40.1%) were male and 149 634 (59.9%) were female. Parameters were measured by Sysmex XN 2000 and 3000 analyzers. The patients were classified according to gender and age groups. In the total population and in each subgroup, the healthy group was selected by Bhattacharya procedure and the reference ranges of NLR, LMR, PLR and MPV were determined.
RESULTS:Patients were divided into 8 groups according to age (1-10, 11-14, 15-20, 21-29, 30-39, 40-49, 50-64,and over 65 years). In the total population reference ranges were found as1.12-6.21, 8.71-11.97,0.30-2.41,39.9-156 for LMR,MPV,NLR and PLR, respectively. Reference ranges for each age range and sex were also evaluated. NLR was higher in females than males except for patients over 65 years of age. Similar results were found for MPV in both sexes and in all age groups. LMR was found to be higher in females in all age groups, but this difference was increased between 15-20 years and over 50 years. PLR was found to be higher in females than males in most age groups.
CONCLUSIONS:Different reference intervals may be used according to gender and different age groups for LMR,MPV,NLR and PLR.
Keywords: Neutrophil, Lymphocyte, Monocyte, Platelet, Inflammation

P-182
Reference values for serum levels of vitamin B12 and folic acid in an adult population

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OBJECTIVES:The aim of this study was to establish reference intervals according to laboratory data in a population and to assess the vitamin B12 (B12) and folate status related to reference intervals for all age and sex groups.
MATERIALS and METHODS:The results were obtained retrospectively from the laboratory information system of Balıkesir State Hospital. The 20318 patients (70.2 % for female, 29.8% for male) between 18- 80 ages were selected. The ages groups of the patient was separated into six subgroups (18–30, 31–40, 41–50, 51–60, 61–70 and 71-80 years). B12 and folate concentrations were measured by ARCHITECT i2000sr (Abbott Diagnostics, Abbott Park, IL, USA) autoanalyzer. Extreme values were excluded by using IBM SPSS. The central %95 reference intervals were calculated using non-parametric method.
RESULTS:The results of 20850 patients for B12 and 14183 for folate were evaluated. The mean+SD years of patients for B12 and folate were 48.9±16.3 and 49.7±16.6, respectively. Mean+SD concentrations of B12 and folate were 298±108 pg/mL and 6.15±2.78 ng/mL, respectively. 95% reference intervals were calculated to 144-536 pg/mL for vitamin B12 and 2.3-14.6 ng/mL for folate. There are statistically significant differences between female and male for B12 and folate. There is a significant difference between the age groups for folate, but there is not a significant difference for B12 concentrations.
CONCLUSIONS:In this study was found differences between the reference ranges recommended by the manufacturer and the reference ranges of our own population. Our results indicate that is important to determine the true reference range.
Keywords: Vitamin B12, Folate, Reference range, Laboratory data, Turkiye

P-183
Review of repeated anti-TPO test requests

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OBJECTIVES:Serum anti-TPO measurements are useful in the diagnosis of autoimmune thyroid diseases and are often sufficient by themselves. Elevated levels are seen in postpartum thyroiditis and Graves' disease as well as Hashimoto's thyroiditis. There isn't any relationship between anti-TPO levels and thyroid function. In the follow-up of patients with Hashimoto's thyroiditis who had high anti-TPO levels, we aimed to compare whether the frequency of recurrent anti-TPO requests decreases with the measures taken.
MATERIALS and METHODS:Anti-TPO test is performed by chemiluminescence method on Advia Centaur XPT (Siemens) analyzer in our laboratory. The anti-TPO tests that were studied in 2017 and 2018 from the LIS were examined.
RESULTS:16,060 anti-TPO tests were conducted in 2017 and 14,130 in 2018. The number of patients who underwent 3 or more anti-TPO tests in 2017 was 223 (789 tests), while in 2018 there were 59 patients (181 tests). While anti-TPO

levels were high in 98 (44%) of the patients who underwent titer monitoring in 2017, it was found to be high in 40 patients (68%) in 2018.
CONCLUSIONS:The necessity of antibody titer monitoring is controversial in patients who have high anti-TPO levels with Hashimoto thyroiditis. Repetitive anti-TPO orderings in diagnosed autoimmune thyroid patients are examples of unnecessary testing. We think that it would be beneficial to display a warning message during the test request in patients with a high anti-TPO level, as well as a time limit test. The collaboration with the clinics that want this test the most has led to a reduction in unnecessary anti-TPO orderings.
Keywords: anti-TPO, Hashimoto tiroiditis, unnecessary test request

P-184
Laboratory data of subclinical hypothyroidism and hyperthyroidism

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OBJECTIVES:To evaluate laboratory data of subclinical hypothyroidism (S-HYPO) and subclinical hyperthyroidism (S-HYPER) on outpatients, frequencies, gender and age distribution and causes.
MATERIALS and METHODS:We performed an epidemiologic study including 144 outpatients, 21 men and 123 women, mean age 50.11yr, for 4 months, with thyroid-stimulating hormone (TSH) outside reference range. Serums of these patients were stored in freezing and tested for anti-thyroglobulin antibodies by ELISA.
RESULTS:We have done 599 TSH tests, 144 cases had TSH outside reference range and 415had normal levels. S-HYPO has a frequency of 12.5% while S-HYPER has a frequency of 8.4%. Subclinical thyroid diseases are found more often in females with 87% to 13% males. Aged <65yr has a frequency of 84% in S-HYPO and 62% in S-HYPER, while the age group ≥65yr has a frequency of 16% and 38% respectively. We measured 91 outpatients for anti-TG and got 21 positive tests, including 11 tests positive for anti-TPO, autoimmune disease is present on 27.4% of patients, multinodular goiter on 9.4%, iatrogenic cause on 9.4% and for 53.8% of patients we don't have a given cause.
CONCLUSIONS:S-HYPO is more frequent than S-HYPER. The gender distribution gives female dominance, the ratio male/female is 1/6.8. In S-HYPO dominate young ages and in S-HYPER dominate older ages. The mild form of S-HYPO is much more frequent than severe form, approximately 8 times more common. The mild form of S-HYPER is 2 times more frequent than its severe form, on both diseases dominate mild forms respectively with 88.6% and 68%.
Keywords: Thyroid stimulating hormone, anti-TG, hypothyroidism, hyperthyroidism, subclinical

P-185
Neutrophil to lymphocyte ratio and mean platelet volume in adults with hypothyroidism

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OBJECTIVES:In this study, neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) and mean platelet volume (MPV) were investigated in adult patients with hypothyroidism.
MATERIALS and METHODS:The records of 496 hypothyroid patients and 5677 euthyroid healthy individuals were compared from the laboratory information system between 10 July 2018 and 09 April 2019.
RESULTS:In the hypothyroid group, free triiodothyronine (f T3), leukocyte, neutrophil and NLR values were lower, thyrotropin (TSH), platelet, PLR, MPV values were higher, free thyroxine (f T4) and lymphocyte values were similar when compared with the euthyroid healthy group.
CONCLUSIONS:In adults with hypothyroidism, platelet count, PLR and MPV values are higher than euthyroid healthy individuals, while leukocyte, neutrophil and NLR levels are low and lymphocyte count is similar.
Keywords: Hypothyroidism, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, mean platelet volume

P-186
Synergistic combination of vorinostat with curcumin induces apoptosis on B-CPAP cells

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OBJECTIVES:Drugs that inhibit histone deacetylase (HDAC) activity show anti-tumor effect in many studies.Vorinostat (SAHA) has numerous applications, including inhibition of malignant cells growth.Curcumin, naturally occurring polyphenol thought to be the newest member of HDAC with potential pro-apoptotic properties.The purpose of the presented study was to investigate the apoptotic effects of Curcumin in combination with Vorinostat on the Papillary Thyroid Cancer (PCT) cells.
MATERIALS and METHODS:Experimental study performed with cell culture from BCPAP cell line.Firstly, Cell viability was assessed using MTT assay following treatment with Curcumin and/or SAHA for 24, 48h.Calcusyn assay used for determination of synergistic dosages of the agents.Apoptotic effects of these agents were marked by Annexin V apoptosis assay and detected by Flow Cytometry.Data were analyzed by the GraphpadPrism statistical program.Values represent the mean±SD (n=3).
RESULTS:According to MTT assay, IC50 values at 48 hour was found 20.97μM and 0.91 μM for Curcumin and SAHA, respectively.Combination treatment of the agents showed markedly synergistic effects (CI=0.891).Synergistic concentrations (9.33 μM for Curcumin, 0.40 μM for (SAHA) were used for later experiments.Curcumin and SAHA alone induced apoptosis at IC50 values while their combination at lower dosages induced synergistic effects.
CONCLUSIONS:The experimental evidence from this study suggests that combination of Vorinostat and polyphenol Curcumin shows synergistic effect which induces apoptosis on PCT cells.Combination of Curcumin and SAHA may be the subject of further study in animal models to determine doses which can exert significant effects in PCT cells and can enhance the therapeutic effect.
Keywords: Curcumin, Vorinostat, BCPAP, Synergism, Apoptosis

P-187
Evaluating the difference of cytotoxicity tests after DMSO exposure in L929 cells

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OBJECTIVES:Cell based cytotoxicity studies aim to investigate the cellular effects of various chemicals. Dose efficiency may change according to the cell type and also the test chosen. DMSO (dimethy sulfoxide) is a widely used compound that cause cellular death. We aimed to compare 4 different cell cytotoxicity tests for their sensibility in the L929 fibroblast cell line.
MATERIALS and METHODS:L929 cells were maintained in Dulbecco's Modified Eagle Medium Supplemented with 10 % fetal bovine serum at 37 °C and 5 % CO2 in a humidified incubator. DMSO concentrations were 0.05%, 0.5%, 1%, 2 % and cells were incubated for 24 h and 48 h. Cytotoxicity was determined with lactate dehydrogenase leakage assay (LDH), neutral red assay (NR), methyl tetrazolium (MTT) assay and crystal violet assay (CV).
RESULTS:Most sensitive result was obtained from CV test but the results were compatible with MTT and LDH assay results. In the comparison of MTT and LDH, LDH results showed higher selectivity for membrane damaged cells. On the contrary, NR assay results showed low sensitivity when compared to other test for all concentrations.
CONCLUSIONS:In the present study, we analyzed four different test for their

efficiency in the evaluation of cytotoxicity according to their mode of action. For L929 cells, CV is most convenient method for evaluating DMSO toxicity. For each cell line it is necessary to begin with the determination of chosing suitable cytotoxicity test in order to increase the accuracy of the work.
Keywords: Cytotoxicity, DMSO, MTT, LDH, NR

P-188
Triazole fungucide flusilazole induced cytotoxicity in SerW3 cells

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OBJECTIVES:Flusilazole is an organosilicon compound, a triazole fungucide which is used for protection of crops. Its presence was reported in cereals and cereal based products. Flusilazole was reported to cause leydig cell tumor in mice for 2 weeks exposure, and testosterone and androstenedione levels of leydig cells decreased in response to flusilazole doses. However no report was exist on Sertoli cells. Sertoli cells have important role in spermatogenesis, two adjacent Sertoli cells compose Sertoli cell barrier. The aim of the study is to reveal possible cytotoxic effects of flusilazole on SerW3 cells mimicking in vitro Sertoli cell.
MATERIALS and METHODS:SerW3 cells (17 days old rat Sertoli cell) were cultured and flusilazole was exposed at concentrations of 0, 25, 100 and 200 µM for 24 hours. MTT and acridine orange/propidium iodide cell viability assays were performed in response to flusilazole in SerW3 cells. Additionally, Sudan Black B staining was performed for lipid droplet detection quantitatively and also examined under light microscope.
RESULTS:Flusilazole treatment caused decreases in cell viability in a dose-dependent manner according to MTT assay results. Acridine orange/Propidium iodide cell viability assay revealed that flusilazole induced apoptotic cell death at high doses. Sudan Black B staining results showed that lipid droplets of the SerW3 cells decreased in response to flusilazole concentrations.
CONCLUSIONS:Results of the study revealed that azole based fungucide flusilazole induced cytotoxicity as well as it caused decreases in lipid droplet accumulation which is essential for Sertoli cell function. This research was financially supported by Hacettepe University, Scientific Research Projects Coordination Unit (Project No: FHD-2018-17594).
Keywords: Flusilazole, cytotoxicity, SerW3 cells

P-190
Investigation of combine effects of propylparaben and methylparaben on pituitary-adrenal axis in male rats

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OBJECTIVES:Propyl paraben and methyl paraben, which are generally preferred in combination, are chemicals that are used as preservatives in many pharmaceutical, food and cosmetic products. Therefore, we are frequently exposed to parabens known to have endocrine disrupting effects in our daily lives. The purpose of the study is to investigate the endocrine disrupting effect of methyl paraben and propyl paraben on the pituitary-adrenal axis.
MATERIALS and METHODS:In this study, 6 experimental groups were designed as 3 control groups (negative, oil and positive control 50 mg/kg bw/day Bisphenol A) and 3 treatment groups (10, 100 and 500 mg/kg bw/day) by mixing 1:1 ratio of methyl paraben and propyl paraben. Doses were administered to the 42-day-old male rats by oral gavage for 30 days.
RESULTS:At the end of the experiment, adrenocorticotrophic hormone, cortisol, aldosterone, androsterone, dihydrotestosterone hormone levels and biochemical values were measured in serum samples. Histopathological effects of pituitary, adrenal glands and liver, kidney tissues which are important in metabolism of toxic substances were investigated.
CONCLUSIONS:In histopathological findings, degeneration, congestion and edema were detected in the tissues. Also, serum cortisol, aldosterone, adrenocorticotrophic hormone and androsterone levels increased in 100 mg/kg bw/day MP+PP and 50 mg/kg bw/day BPA, serum dihydrotestosterone hormone increased in 10 mg/kg bw/day MP+PP, 500 mg/kg bw/day MP+PP and 50 mg/kg bw/day BPA and serum triglyceride levels increased in 100 mg / kg bw/day MP+PP dose group, results showed that propyl paraben and methyl paraben have an effect on HPA axis hormonal activity.
The authors thank to Scientific Research Unit of Hacettepe University (Project No: FHD-2018-17085).
Keywords: Propyl paraben, methyl paraben, endocrine disruptors, male rats

P-191
Urine iodine deficiency in pregnant women living in Sivas

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OBJECTIVES:Trace elements are defined as chemicals present in minimal quantities.Some of these elements including iodine, iron, and selenium are also entitled to micronutrients.Iodine is an essential component of the thyroid hormones.Therefore, various metabolic and neurologic disorders have been associated with iodine deficiency (ID).ID is a threat throughout the lifecycle. The effects of inadequate iodine intake change according to the stage of lifecycle. The study aimed to assess the iodine status and maternal thyroid function in the pregnant women in Sivas that is a city in central Turkey.
MATERIALS and METHODS:This study was performed with the collaboration of Cumhuriyet University Department of Biochemistry and Department of Obstetrics and Gynecology and Sivas Numune Hospital Department of Obstetrics and Gynecology between 2015 and 2016.One Hundred-ninety-three pregnant women in their second trimester who attended the hospital for routine antenatal care were included this study.Morning spot urine samples were collected in deiodized test tubes.Urine iodine levels were determined by colorimetric modified Sandell Kolthoff method.
RESULTS:The range of gestation week was 5th-13th in all locations. Median gestation weeks were 8 weeks 2 day, 8 weeks, 8 weeks 4 day,10 weeks, 7 weeks 2 day and 8 weeks in Sivas Centre,Şarkışla,Suşehri,Gürün,Divriği, and Kangal,respectively.Median ID levels of pregnant women living in Şarkışla,Suşehri,Gürün,Divriği,and Kangal indicated inadequate iodine intake.
CONCLUSIONS:Our results indicated that iodine deficiency is a significant problem in Sivas. Therefore, there is a need policy such as iodine prophylaxis for women living in Sivas to eliminate this problem. Finally, these are only preliminary findings, and further investigations with larger samples are warranted.
Keywords: Urine iodine, Sivas, Pregnant women

P-192
Changed iron homeostasis in sleeping apnea patients

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OBJECTIVES:Obstructive sleep apnea syndrome (OSA) is defined as a combination of symptoms as a result of intermittent, recurrent constraint and / or complete airway overhead airway overflow (sleep disturbance). OSA is associated with the development of insulin resistance, arterial hypertension,

metabolic syndrome, systemic atherosclerosis and increased cardiovascular risk.
MATERIALS and METHODS:40 patients with OSA were included. Their results were compared to sex and age matched healthy control. CBC, serum iron, ferritin, hsCRP, hepcidin, homocysteine and vitamin B12 were measured in the included groups. Intima media thickness (IMT) and Flow mediated dilatation (FMD) were used for atherosclerotic changes evaluation.
RESULTS:We found increased serum hepcidin levels in OSA patients with IMT and FMD changes (121.7 ± 11.9 µg/L) compared to control group (20.4 ± 1.8 µg/L); P<0.005. A positive correlation was found in OSA patients with atherosclerotic changes between IMT and FMD to serum hepcidin levels (r=0.859, r=0.871, resp.; P<0.05). Serum hepcidin correlates positively to homocysteine and vitamin B12 in OSA patients (r=0.902, r=0.911, resp.; P<0.005).
CONCLUSIONS:Brain-vascular disease risk factors are connected to obstructive sleep apnea syndrome. Disregulation of iron homeostasis is one of the main risk atherogenesis factors. Early hepcidin quantification might predict an atherosclerosis occurrence in OSA patients, which might be very important for better clinical diagnosis and practice.
Acknowledgements: This project is sponsored by MU-Sofia, as part of Grant Д-52/2018.
Keywords: sleep apnea, iron, hepcidin

P-193
Comparison of urine analyzers LabUMat2-with-UriSed2 and Sysmex UC-3500/UF-5000

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OBJECTIVES:Instrument method comparison experiments are based on the comparison of the results obtained by analyzing the samples with the new test method and the previously accepted method. In this study, we aimed to compare LabUMat2-with-UriSed2 and Sysmex UC-3500/UF-5000 analyzers in urine examination.
MATERIALS and METHODS:The urine samples of thirty patients were studied on LabUMat2-with-UriSed2 and Sysmex UC-3500/UF-5000 analyzers. Density, pH, RBC, WBC, hyaline casts, yeast, squamous epithelial cells, non-squamous epithelial cells were recorded for each patient. The distribution of the data was examined and the correlations were checked.
RESULTS:LabUMat2-with-UriSed2 and Sysmex UC-3500/UF-5000 analyzers showed that the density, pH, RBC, WBC, squamous epithelial cells and non-squamous epithelial cells correlated significantly ($r = 0.803$, $p = <0.001$; $r = 0.950$, $p = < 0.001$; $r = 0.730$, $p = <0.001$; $r = 0.695$, $p = <0.001$; $r = 0.437$, $p = 0.016$; $r = 0.377$, $p = 0.040$, respectively), but hyaline casts and yeast cells showed no correlation ($p > 0.05$) statistically.
CONCLUSIONS:The results obtained for density, pH, RBC, WBC, squamous epithelial cells and non-squamous epithelial cells were compatible with each other, however hyaline casts and yeast cells were not. Hence, it maybe considered that manual microscobic confirmation can be beneficial for pathological urine samples.
Keywords: urinalysis,correlation,analyzer comparison

P-194
Evaluation of results with the use of autoverification ın urinanalysis

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OBJECTIVES:Urinalysis is a chemical and microscopic analysis of urine and is frequently performed in clinical laboratories. Urinalysis is often required for evaluation of hematuria and urinary tract infections. Autoverification is a process of using an algorithm-based program where criteria are defined and approving the samples that meet all criteria without user intervention. The aim of this study is to evaluate the results with the use of autoverification in urine autoanalyzer in our laboratory.
MATERIALS and METHODS:This study was performed between May and August 2019 in Beckman Coulter IQ 200 Elite urine autoanalyser. The rules were defined by the iware program to the urine autoanalyzer. While the results that were in compliance with all the rules were automatically verified, the test results that did not follow the rules were taken to perform manual operation on the analyzer.
RESULTS:When the three-month period was examined, it was observed that 28113 samples analyzed in total and 11371 of these samples were autoverified. The rate of autoverified results was 40.4%.
CONCLUSIONS:With the use of the autoverification, it was observed that a significant part of the results did not require user intervention so laboratory technicians can spend more time on incompatible results of chemical and microscopic analysis. Thus, it is obvious that Turn Around Time will be reduced. We believe that the autoverification will alleviate the increased workload of clinical laboratories and save the energy and time that laboratory experts can allow time to other clinical studies.
Keywords: Autoverification, Urinanalysis

P-195
Comparison of Iris iQ200 urine analyzer performance and manual microscopy in examination of urine sediments

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OBJECTIVES:Complete urinalysis is one of the most widely used tests in clinical laboratories today. Urine analysis is an inexpensive, time-saving, easily applicable diagnostic tool that provides important information about kidney function. Although manual analysis applications are standardized, conventional microscopy of urine sediment is labor intensive, time-consuming, uncertain, and varies widely. Automated urine analysis saves time and labor. The aim of this study was to evaluate the performance of urine analyzer Iris iQ200, which has an image-based analysis system using a video camera, by comparing it with manual microscopy.
MATERIALS and METHODS:Freshly collected urines of 30 patients who presented to Gaziantep University Medical Faculty and whose urine examinations were requested by the physicians were studied. After chemical analysis and microscopic examination with the Iris iQ200 autoanalyzer, the remaining urine sample was centrifuged at 1500 rpm (400g) for 5 minutes, and the resulting sediment was evaluated for erythrocytes, leukocytes and crystals using manual microscopy.
RESULTS:The consistence between Iris iQ200 analyzer and Pearson correlation analysis was used to assess manual microscopic results. In the comparison of the two methods, the erythrocyte correlation coefficient was r=0.999, the crystal correlation coefficient was r=0.495, and the leukocyte correlation coefficient was r=0.725.
CONCLUSIONS:Between the two methods, high level of consistency in the erythrocyte analysis, moderate level of consistency in the crystal analysis, high level of consistency in the leukocyte analysis were observed. Compared to manual microscopy, the Iris iQ200 instrument tested in this study showed satisfactory analytical performances for erythrocytes and leukocytes.
Keywords: Iris Q200; automated urine sediment analyzer; urine microscopy; urine sediment

P-196
Evaluation of correlation between 24-hour urine protein level and spot urine protein-to-creatinin ratio

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OBJECTIVES:Measurement of 24-h protein excretion is the reference method for determination of urinary protein excretion. Because of patient incompliance, protein/creatinin ratio (P/Cr) in spot urine is commonly used. In this study, 24-h urine specimen protein levels and P/Cr ratios in spot urine were compared.

MATERIALS and METHODS:Retrospectively, datas of outpatients and inpatients included between 1st January 2018 and 1st May 2019. Urine protein was measured with colorimetric method by Beckman Coulter 5800 analyzer. Urine creatinine was measured with Jaffe method by Beckman Coulter 5800 analyzer. Cases (n=157) were separated into 3 groups according to P/Cr ratios 0-0.2 (n=71), 0.2-2 (n=56), >2 (n=30)). Kolmogorov-Smirnov test is used for homogenity of groups. Due to p<0.05 was applied Spearman correlation analysis. Descriptive statistics and correlation analysis were calculated by SPSS version 22 statistical programme.
RESULTS:In each group, we compared spot urine P/Cr ratios with 24-h urine protein levels. In first group, (P/Cr = 0-0.2) 24-h urine protein results (median:109.59; 25th-75thpercentil:71.2-143.0) and spot urine P/Cr (median:0.09, 25th-75thpercentil:0.07-0.14) were calculated. Spearman (r=0.502, p=0.0001) test was applied. In second group, (P/Cr=0.2-2) 24-h urine protein results (median: 693.20, 25th-75thpercentil:293.91-1145.38) and spot urine P/Cr (median:0.67, 25th-75th:0.42- 1.13) were calculated. Spearman (r=0.705, p=0.0001) test was applied. In third group, (P/Cr = >2) 24-h urine protein results (median: 4504.66, 25th-75th percentil: 2259.40- 6192.90)and spot urine P/Cr(median: 4.50, 25th-75th percentil:3.00- 7.76) were calculated. Spearman (r=0.427, p=0.019) test was applied.
CONCLUSIONS:24-h urine protein levels are significantly correlated to spot urine P/Cr ratios.
Keywords: proteinuria, protein/creatinin ratio, 24-h urine protein

P-197
Comparison of phase contrast microscopy with light microscopy in evaluation of urinary sediment

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OBJECTIVES:It is known that light microscope (LM) used in routine studies in urine sediment analysis has some limitations. For example, some shaped elements with low refractive index in urine may not be distinguished from the ground.Therefore, as an alternative approach, phase contrast microscopes (FCM) can be used to better distinguish.The advantage of FCM is that it is sufficient to examine and evaluate the morphological and physical properties of living organisms.In this study, we aimed to compare the analytical performances of FCM and LM in routine urine analysis.
MATERIALS and METHODS:130 samples were studied, over 18 years of age. Samples prepared within 2 hours and examined under microscope. Intense and turbid urines with low amounts and excessive hematurics not included. Urine sedimentation tubes were collected in 10 mL samples and centrifuged at 400 g for 4 minutes.Then 9 mL of the supernatant was carefully decanted and remaining amount examinated.
RESULTS:We found the differences in terms of image and noticed that some structures are more clearly seen in FCM and these structures can't be selected under LM. FCM was found to be more advantageous in determining cell morphology and a positive correlation was found between the two methods in all parameters.
CONCLUSIONS:Some structures can't be detected under LM. Therefore, examination of sediment with FCM increases the efficiency of diagnosis and treatment and can be used in clinical laboratories instead of LM,also can be incorporated into newly manufactured devices.Since there are no publications comparingLM and FCM in urine sediment, we recommend further studies.
Keywords: phase contrast microscopes (FCM),light microscope (LM),urine sediment

P-198
Comparison of strip and nephelometric method in the screening of urine microalbumin

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OBJECTIVES:Early diagnosis of renal abnormalities is essential to prevent the progression to irreversible phases. The earliest sign of the renal disease is the presence of microalbumin in the urine, which can detect via several methods. In this study, we compared the strip method with the immunonephelometric method. Our aim was to investigate the reliability of fast and low-cost first-step methods for microalbumin screening.
MATERIALS and METHODS:We analysed the urine samples with the albuminuria test request between June - July 2018. Firstly, urine samples were analysed quantitatively via an immunonephelometric method with the Roche Cobas 6000 instrument, following a second semi-quantitative analyser with Sysmex UC-3500 urine analyser. Results were compared using Spearman correlation analysis.
RESULTS:A strong correlation was found between two analysers. Correlation was, r:0.831 (n: 86; p <0.01) and r:0.872 (n: 105; p <0.01) for albumin and creatinine, respectively. The coefficient of determination was detected r2=0.83 and r2=0.738, for the levels of urine albumin and creatinine, respectively. As the clinical decision-making limit for albuminuria considered as 30 mg/dL, sensitivity, specificity, negative predictive value and positive predictive values for Sysmex UC-3500 were detected as 100%, 26%, 100%, and 57%, respectively.
CONCLUSIONS:In this study, we showed that semi-quantitative systems may be an alternative for the first step screening with positive correlation and 100% NPV sensitivity, despite having a simpler technology. Urine strips may be a good option in clinical laboratories because of the low costs and rapid test results.
Keywords: Microalbumin, Sysmex UC-3500, strip, urinalysis, proteinuria

P-199
Inflammation biomarkers in patients classified in accordance with serum B12 levels

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OBJECTIVES:In the general population, vitamin B12 deficiency is a relatively common finding. We aimed to investigate the possible difference in inflammation markers between three categories based on serum B12 level.
MATERIALS and METHODS:Male patients' (18-60 years) one year data was classified according to serum vitamin B12 levels (DxI800, Beckman Coulter Inc.); Group-1: B12 < 146 ng/L, Group-2: 146-180 ng/L, Group-3: 181-348 ng/L. Patients with white blood cell (WBC) over 15.0 × 10^9/L were excluded. We also excluded some clinics where test requests were made: intensive care units, oncology, emergency, infectious diseases, nephrology departments. After eliminating the extreme values (N=2,111) by Horn algorithm, backward linear regression analysis was performed for the remaining 2,485 patients.
RESULTS:In Group3, we had lower neutrophil/lymphocyte ratio than Group 1 (p<0.05). Regression analysis revealed an independent relationship between vitamin B12 and WBC, neutrophil counts (standardized regression coefficients, βWBC = 0.108, p<0.010 and βNeutrophil = -0.101, p=0.016, respectively).
CONCLUSIONS:Recently, it was reported that the B vitamins are required for cytotoxic cellular immunity and modulating T cell responses. Our B12 levels are positively correlated with WBC (possibly lymphocyte-induced) and negatively correlated with neutrophil counts; this finding may support the role of Vitamin B12 as an immunomodulator. One of the major problems is knowing what the reference range of vitamin B12 is. Considering B12 levels in evaluation of immune status may be helpful for clinical approach.
Keywords: vitamin B12, neutrophil, lymphocyte, inflammation.

P-201

Ultrasensitive blood sugar monitoring by mobile phone ıntegrated, reusable BorA-MeTiN composite sensors

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OBJECTIVES: Conventional glucometer strips are modified by glucose oxidase for glucose detection which is open to effects of by oxygen concentrations, pH, temperature and other physical parameters. Besides they are not reusable which limits their cost-effectiveness. In this study, we used molecularly (glucose) imprinted Boronic Acid (BorA) Mesoporous Titanium Nanoparticles (MeTiN) to achieve accurate and cost-effective results in blood sugar self-monitoring.
MATERIALS and METHODS: Glucose, Aminophenyl BorA (APBA) and MeTiN(1:1:1, w/w) solution was dropped (50uL) on screen printed platinium electrodes. Afterwards, 700 mV potential was applied for 20 minutes to polymerize BorA monomers electrochemically. Then, pH=3 HCl solution was applied to remove glucose from glucose-imprinted BorA-MeTiN composite sensor to reveal glucose imprinted cavities for rebinding. The sensor optimization was tested by electrochemical impedance spectroscopy (EIS).
RESULTS: According to the optimization studies, electrode LOD was calculated as 0.52 ng/mL glucose concentrations. Linearity of the electrode was between 1-2000 ng/mL glucose levels. Regression coefficient value was obtained from 18 calibration curves of the same electrode as R2=0.9873±0.0102. Electrodes were connected to a mobile phone via Arduino open circuit system to assess the compatibility of the sensor. With a software and mobile phone, we detected the 100ng/mL glucose levels in serum samples with %12.44 error.
CONCLUSIONS: MeTiN supported APBA polymerization increased the sensitivity and selectivity of blood glucose determination. We used one electrode for 18 times. The stability of the MeTiN increased the molecularly imprinted polymer stability which enabled multiple usage. Thus, the novel sensor has the potential to be reusable and mobile phone integrated.
Keywords: glucose, molecular imprinting, boronic acid, titanium nanoparticle, impedance

P-202

Serum prolactin and ferritin levels in particular autoimmune disease

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OBJECTIVES:Prolactin (PRL) is a polypeptide hormone, mainly synthesized in anterior pituitary gland. Ferritin is a 24-subunit protein, which has a mainly function as an iron-storage protein. The purpose of this study was to classify high PRL and ferritin levels in patient with different disease, and to evaluate their values in such autoimmune disease.
MATERIALS and METHODS:The patients who applied to our university hospital, between Jan2018-Jul2019, and measured serum ferritin or PRL levels were included into the study. It was classified 2909 patients who had ferritin levels> 400 µg/L, and then they were divided into 5-subgroups between 400->7000 µg/L. For PRL, 1048 patients were classified as PRL>19 ng/ml for males, and PRL>26 ng/ml for females, and then they were divided into 6-subgroups between 50->5000 ng/mL. Two-site Immunoenzymatic assay (Beckman Coulter, Inc) and chemiluminescent-microparticle immunoassay (CMIA) (Abbott, Diagnostics) were used for serum ferritin and prolactin assays, respectively.
RESULTS:Patients with hemophagocytic-lymphohistiocytosis had the highest ferritin levels (>20000 µg/L), followed by severe-combined-immunodeficiency (11544 µg/L). Such disease, systemic lupus erythematosus, polymyositis, Crohn's disease, rheumatoid arthritis, Myasthenia Gravis, dermatomyositis was more likely to be hyper ferritin-affected diseases when compared with the general population. The highest serum PRL levels were observed in neoplasm(n=192), anemia(n=121) and psychiatric disorders(n=79). Serum PRL was also high in autoimmune thyroiditis and systemic-lupus-erythematosus.
CONCLUSIONS:Our results showed increased serum PRL and ferritin levels in autoimmune diseases. This may have clinical significance. Ferritin may induce complete activation of the immune response and PRL may play a role in the maturation of T lymphocytes.
Keywords: Autoimmune diseases, ferritin, prolactin

P-205

The combined effects of urea-based herbicide linuron and elevated temperature on biological responses and stress biomarkers

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OBJECTIVES:Linuron is a widely used urea-based herbicide that has endocrine disruptor activity. This study aimed to further elucidate the potential effects of linuron on reproductive, biochemical and hematological biomarkers at different temperature conditions.
MATERIALS and METHODS:The combined effects of linuron and elevated temperature were studied on Cyprinus carpio. Carp were acclimated to two different temperatures (22 °C and 28 °C) for 15 days. Then, fish were exposed for 96 hours to linuron at environmentally relevant concentrations of 10 and 100µg/L at 22 °C and at elevated water temperatures (28 °C).
RESULTS:We found that combined temperature increase and pesticide exposure affected the biological responses in C. carpio. Linuron caused an elevation in hematocrit level while it did not change hemoglobin concentration. An increase in the AST enzyme activity was determined. The herbicide caused persistent decrease in cortisol level and ALT enzyme activity. In addition, linuron exposure caused remarkable alterations in estrogen and testosterone levels.
CONCLUSIONS:This study indicates that the combined effects of linuron and elevated temperature induced the steroidogenesis, hematological and biochemical biomarkers. The results of this study could be used to assess the effects of environmentally relevant concentrations of pesticides.
Keywords: Pesticide toxicity, climate change, endocrine disruption

P-206

Investigation of the effect of CRY1 on nucleotide excision repair in mouse embryonic fibroblasts

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OBJECTIVES: The circadian rhythm (CR) is the internal timing sytem which is considered to affect every biochemical, physiological, behavioral process and is affected by them. It is generated and controlled by positive (CLOCK (or NPAS2)-BMAL1/2) and negative (PERIOD (Per1/2/3)-CRYPTOCHROME (CRY1/2)) feedback loops. Another accessory negative loop involving NR1D1/2 (REV-ERBα/β) has also been reported. Nucleotide excision repair (NER) is the most general repair mechanism for removing bulky lesions from the genome, and defective NER is implicated in various pathological conditions including cancer and neurodegenerative diseases. It is the sole pathway to repair UV induced pyrimidine dimers and [6,4]-photoproducts. It was shown that NER activity has a CR in mouse brain and the core NER protein XPA oscillates at the same phase with Bmal1 and anti-phase with Crytopchrome1, leading the hypothesis that there is a link between CR and NER. In this study, the effect of CRY1 on the repair of [6,4]-photoproducts in mouse embryonic cells lacking the negative loop of the CR was investigated.
MATERIALS and METHODS: Per1/2(-/-) cells were produced by using TALEN

genome editing, and nr1d1/2, Cry1/2(-/-) cells were prepared from them by the CRISPR/Cas9 system. CRY1(+/+) cells were the ancestor of Per1/2(-/-),nr1d1/2(/-),Cry2(-/-) lines. Two cell lines (Cry1(+/+) and Cry1(-/-), both Cry2,Per1/2, nr1d1/2 KO) were exposed to ultraviolet-C (25 J/m^2) radiation, and the NER was evaluated for each group by Immunoslot Blot. The data were analyzed with ImageQuant software.
RESULTS: Our data show that CRY1 expression does not have an effect on the repair of [6,4] photoproducts when cell lines were exposed to 25 J/m^2 UV-C.
CONCLUSIONS: More studies are needed to clarify the possible role of specific circadian rhythm components on NER.
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Technological Research Council of Turkey (TÜBİTAK) (G.K.-E.); and the Hacettepe University Scientific
Research Projects Coordination Unit (G.K.-E.)
Keywords: CRY1, Circadian rhythm, Nucleotide excision repair, Immunoslot blot, Ultraviolet radiation

P-207
Investigation of Antioxidant activity in Plants Commonly Grown in Kahramanmaraş Region

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OBJECTIVES:The use of plants to cure several kinds of human diseases has a long history. Various parts of plants such as leaf, stem, bark, root, etc. are being used to prevent, allay symptoms or revert abnormalities back to normal. Although there are scientific studies about the antioxidant activities of some plants is limited. In this study, it was made to determine the antioxidant activities of different plants in commonly grown in Kahramanmaraş region which known to be used among the public.
MATERIALS and METHODS:In our study, antioxidant activities of three different plants belonging to different families in Kahramanmaraş region were investigated. for this purpose, we studied menengiç (Pistacia terebinthus L.), ışgın (Rheum ribes L.) and çiriş otu (Asphodelus aestivus). Firstly, the plants cut into small pieces with a knife. Then, the plants were homogenized with three volumes of ice-cold 1.15 % KCI. The activities of antioxidant enzymes and malondialdehyde (MDA) levels were measured in the supernatant obtained from centrifugation at 14.000 rpm. The activities of superoxide dismutase (SOD) and catalase (CAT) as antioxidant enzymes and MDA levels in plants were masured as spectrophotometric.
RESULTS:While the highest CAT was found to be the maximum in Rheum ribes, It was found as lowest in Asphodelus aestivus ($p<0.05$). Also, SOD activity was found as highest in Rheum ribes ($p<0.05$). However, the lowest SOD activity was found in Pistacia terebinthus ($p<0.05$). While the levels of MDA were found as highest in Rheum ribes, the lowest MDA levels were found in Asphodelus aestivus ($p<0.05$).
CONCLUSIONS:Our results indicated that Rheum ribes has the highest antioxidant enzyme capacity due to the highest metabolic activity of all them.
Keywords: Rheum ribes. Asphodelus aestivus, Pistacia terebinthus.

P-208
Resveratrol, a natural antioxidant, attenuates liver ischemia/reperfusion injury in rats

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OBJECTIVES:Oxidative stress mediators are believed to contribute to the liver ischemia/reperfusion (I/R) injury. Resveratrol, a polyphenol found in grapes, is shown to be a strong antioxidant in various tissues, with a property of an estrogen-receptor agonist. Therefore, we investigated the effects of resveratrol on oxidative injury in the liver.
MATERIALS and METHODS:Female Wistar rats were randomly allocated into three groups (n = 8, each). The sham group (as control) was only subjected to surgical procedures, while other animals were subjected to liver ischemia (60 min) and subsequent reperfusion (60 min). One group received resveratrol (15 mg/kg, 0.3 mL/day intraperitoneally) for both 5 days before surgery and 15 min before ischemia (I/R+resveratrol group), while the other was treated intraperitoneally with 0.09 % saline as group (0.3 ml/day) (I/R group). At the end of this experimental, activities of catalase (CAT), superoxide dismutase (SOD) and the levels of malondialdehyde (MDA) were measured as spectrophotometric in liver tissues homogenates.
RESULTS:In the I/R rat liver, we detected severe tissue injuries ($p<0.001$), the significant increases in the tissue levels of MDA ($p<0.001$), and the decrease in activities of SOD and CAT ($p< 0.001$), compared to the sham control. Resveratrol significantly ameliorated the liver injury, decreased MDA levels to the sham control levels ($p<0.001$). Resveratrol also restored the SOD and CAT activities.
CONCLUSIONS:These results suggest that resveratrol could protect liver tissue against I/R injury with its potent antioxidant properties.
Keywords: Resveratrol, liver ischemia/reperfusion, antioxidants

FULL-TEXT ARTICLES FROM ORAL PRESENTATIONS

Transcriptomic Meta-Analysis in Pancreatic Ductal Adenocarcinoma Reveals Therapeutic Targets and Diagnostic Biomarkers

[Transkriptomik Meta-Analiz ile Pankreatik Duktal Adenokarsinomada Terapötik Hedefler ve Diagnostik Biyobelirteçlerin Belirlenmesi]

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ABSTRACT

BACKGROUND: Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer, which has the highest mortality rate of all solid tumors. The absence of an effective screening process and distinctive symptoms causes a delay in diagnosis. Traditional chemotherapy and curative surgery have limited benefits on patient survival. Enzymes are one of the most important groups of drug targets and are preferred markers for the detection of various diseases.

OBJECTIVES: This study aims to identify up-regulated genes encoding enzymes in PDAC to suggest novel therapeutic targets for more effective treatments to be developed and diagnostic biomarkers for PDAC.

MATERIALS and METHODS: NCBI Gene Expression Omnibus (GEO) was searched for datasets using keywords 'pancreatic ductal adenocarcinoma'. The inclusion criteria were i) Gene expression microarray data, ii) human-derived pancreatic ductal adenocarcinoma tissues and normal pancreatic tissue samples. All data processing and integration procedures were performed using ExAtlas. The false discovery rate is less than 0.05, and the change of gene expression is ≥ 10-fold were considered significant. The up-regulated enzyme-coding genes were detected in the differentially expressed gene list. The identified up-regulation of enzyme-coding genes in PDAC was verified using datasets from TCGA and GTEx projects.

RESULTS: The random effect integrative meta-analysis of five submissions (GSE46234, GSE19280, GSE43795, GSE41368, and GSE71989) containing 24 tumor-normal tissue pairs revealed 22 up-regulated genes, two of which encoding enzymes. The enzyme-coding genes with at least 10-fold differential expression compared to the controls were SULF1 (sulfatase, fold change=22.135) and KYNU (kynureninase, fold change=10.716), in consistence with the results from TCGA and GTEx data.

CONCLUSIONS: The results of this study suggest that sulfatase and kynureninase may have the potential to become diagnostic biomarkers and therapeutic targets for PDAC, which merits further investigation.

Keywords: Pancreatic Ductal Adenocarcinoma, microarray, meta-analysis, enzymes, gene expression

ÖZET

Genel Bilgi: Pankreatik duktal adenokarsinoma (PDA), en yaygın pankreas kanseri türü olup tüm solid tümörlerde en yüksek ölüm oranına sahiptir. Etkili bir tarama prosesinin ve ayırıcı semptomlarının bulunmayışı tanıda geç kalınmasına neden olmaktadır. Geleneksel tedavi yöntemleri ve küratif cerrahinin hastaların yaşam oranlarında sağladığı iyileşme oldukça kısıtlıdır. Enzimler, ilaç hedeflerinin önemli bir grubunu oluşturmakta olup aynı zamanda birçok hastalığın tanısında tercih edilen belirteçlerdir.

Amaç: Bu çalışma, PDA'da daha etkili tedavi yöntemlerinin geliştirilmesi için yeni terapötik hedefler ve diagnostik biyobelirteçler önermek amacıyla enzim-kodlayan ve PDA'da yüksek eksprese olan genleri tanımlamayı amaçlamaktadır.

Gereç ve Yöntemler: NCBI Gene Expression Omnibus (GEO) veri bankası 'pankreatik duktal adenokarsinoma' anahtar kelimesi kullanılarak tarandı. Çalışmanın inklüzyon kriteri; i) Gen ekspresyon microarray datası, ii) insan kaynaklı PDA ve normal pankreatik doku örneklerini içeren çalışmalar olmasıdır. Tüm veri işleme ve entegrasyon prosedürleri ExAtlas kullanılarak yapıldı. Meta-analiz, random effects metodu kullanılarak gerçekleştirildi. FDR oranının 0.05'ten az olması ve gen ifadesindeki değişimin ≥10 kat olması istatistiksel olarak anlamlı kabul edildi. Ekspresyonu yükselmiş olan enzim kodlayan genler ekspresyonları farklılaşmış gen listesinde tespit edildi. PDA'da tanımlanan enzim-kodlayan genlerin yüksek ekspresyonu TCGA ve GTEx projelerinden elde edilen veri setleri kullanılarak doğrulandı.

Bulgular: Totalde beş GEO veri setinden 24 tümör-normal doku çiftinin dahil edildiği rastgele etki bütünleştirici meta-analizi, ile PDA'da 22 yüksek eksprese olan gen belirlenmiştir. Kontrollere kıyasla en az 10 kat diferansiyel ekspresyonu olan enzim kodlayan genler, TCGA ve GTEx verilerinin sonuçları ile uyumlu olduğu bulunan, SULF1 (sülfataz 1, kat değişimi = 22.135) ve KYNU (kinüreninaz, kat değişimi = 10.716) olarak belirlenmiştir.

Sonuç: Bu çalışmanın sonuçları, sülfataz 1 ve kinüreninazın PDA'da diagnostik biyobelirteç ve terapötik hedef olma potansiyellerine sahip olabileceğini göstermektedir.

Anahtar Sözcükler: Pankreatik duktal adenokarsinoma, mikroarray, meta-analiz, enzim, gen ekspresyonu

INTRODUCTION

Pancreatic cancer (PC) is a highly fatal malignancy and the fourth common cause of death from cancer within all types of tumors [1]. Pancreatic ductal adenocarcinoma (PDAC) and its variants are the most common types of pancreatic cancer and constitute more than 90% of all cases [2]. The overall 5-year survival rate for patients with pancreatic cancer remains less than 8% and a one-year survival is around 18% when all stages are combined [1, 3]. Pancreatic cancer is asymptomatic in early stages; thus, the majority of patients with pancreatic cancer present at advanced stages. Besides, the absence of an effective screening process contributes to the resulting delayed diagnosis [4, 5]. Over 50% of patients with pancreatic cancer are diagnosed at the metastatic stage of the disease, and approximately 85% of these patients have a survival rate of less than one year [6]. Patients with metastatic state of the disease cannot benefit from surgical resection improving median overall survival to 11-23 months [7]. Even after radical resection, 60% of the patients experience local and systemic relapse within the first 12 months after curative resection [8], and more than 80% of the patients die of the disease due to local recurrence and/or distant metastasis [9]. Thus, early diagnosis of PDAC remains challenging and current therapeutic modalities are still inadequate in the treatment of the disease. There is an urgent need for the identification of diagnostic biomarkers and the development of novel and effective targeted therapies.

High transcriptome profiling is one of the most utilized approaches in the identification of novel prognostic or diagnostic biomarkers, and the development of more effective therapeutic targets. With the increasing number of studies using high transcriptome profiling, integration of transcriptome data by combining microarray raw data from several studies has become a preferred method to increase sample size, statistical power, and reliability for studies aiming to find novel biomarkers. Several meta-analyses have been conducted to determine molecular and clinical subtypes of pancreatic cancer [10], and to identify novel prognostic [11-14] or diagnostic biomarkers [15]. Using an integrative high transcriptome meta-analysis approach, this study aims to identify up-regulated enzyme-coding genes in PDAC and to suggest novel diagnostic biomarkers and therapeutic targets for the disease.

MATERIAL and METHODS

Selection of Microarray Datasets

NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) was systematically searched for eligible datasets using the keywords 'pancreatic ductal adenocarcinoma'. Inclusion criteria were: *i)* Gene expression microarray

data, *ii)* studies included human-derived pancreatic ductal adenocarcinoma tissues and normal pancreatic tissue samples.

Generation of Gene Expression Matrix Files and Evaluation of Data Quality
All data processing and integration procedures were performed using ExAtlas according to the manual of the software [16]correlation matrix,gene set enrichment,ANOVA,PCA,expected proportion of false positives</keyword></keywords><dates><year>2015</year></dates><accession-num>26223199</accession-num><urls><related-urls><url>https://www.worldscientific.com/doi/abs/10.1142/S0219720015500195</url></related-urls></urls><electronic-resource-num>10.1142/s0219720015500195</electronic-resource-num></record></Cite></EndNote>. The datasets that have been selected for meta-analysis were uploaded to ExAtlas and then gene expression matrix file was generated from each dataset separately. All the extracted data were pre-normalized with the RMA algorithm. Samples, where the correlation of expression of housekeeping genes in the range from 0.5 to 0.95, and the level of standard deviation from the global mean for each set of genes grouped by the average expression is less than 0.3, were considered to be of good quality. Samples of low quality were removed from the dataset.

Standard Meta-Analysis
The meta-analysis was performed using random-effects method [17], which takes into account the variance of heterogeneity between studies. The analysis was performed for each gene symbol and every tumor sample was compared with one of the normal pancreatic tissue samples selected randomly from the same dataset. False discovery rate is less than 0.05, and the change of gene expression is ≥ 10-fold were considered significant. The enzyme-coding up-regulated genes in PDAC compared to the normal pancreatic tissues were reported.

Verification of the results in another dataset
The Gene Expression Profiling Interactive Analysis (GEPIA) online database (http://gepia.cancer-pku.cn/index.html) is an interactive web server for analyzing RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects, using a standard processing pipeline [18]. It provides key interactive and customizable functions such as differential expression analysis between tumor and normal samples. Thus, to verify the results of this study, a boxplot was employed to visualize the expressions of up-regulated enzyme-coding genes identified in this study in pancreatic adenocarcinoma (n=179) and normal pancreatic tissue datasets (n=171) from TCGA and GTEx projects.

RESULTS

Microarray Datasets
A systematic search of the studies was carried out up to September 2019. Gene Expression Omnibus Datasets were searched with the term 'pancreatic ductal adenocarcinoma' and the search results were filtered by selecting organism as homo sapiens, study type as expression profiling by array and attribute name as tissue. As a result, 54 studies were found in GEO Database. It was carefully examined whether these studies met the inclusion criteria of this study. A total of 24 PDAC and 24 normal pancreatic tissue samples from five submissions (GSE46234, GSE19280, GSE43795, GSE41368, and GSE71989) were decided to be eligible for the meta-analysis (Table 1).

Table 1. Eligible public datasets used in the meta-analysis.

Public Datasets	Platform	PDAC (n)	Healthy (n)	PMID
GSE46234	HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	6	2	-
GSE19280	[HG-U133B] Affymetrix Human Genome U133B Array	4	3	23007696
GSE43795	Illumina HumanHT-12 V4.0 expression beadchip	6	5	24072181
GSE41368	[HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]	6	6	24120476
GSE71989	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	13	8	27363020

Up-regulated Enzyme-coding Genes in PDAC
The random effect meta-analysis resulted in 22 differentially over-expressed genes in PDAC compared to the healthy pancreatic tissue samples (Table 2).
The most significantly up-regulated gene in PDAC was POSTN (Periostin) with an approximately 45-fold differential expression compared to the controls. The up-regulated enzyme-coding genes in PDAC were SULF1 (sulfatase 1, fold change=22.135) and KYNU (kynureninase, fold change=10.716).

Table 2: The differentially expressed genes with at least a 10-fold differential expression between pancreatic ductal adenocarcinoma and normal pancreatic tissues. **FDR: False discovery rate*

Gene Symbol	Gene Name	Fold Change Combined	P-Value	FDR*
POSTN	periostin, osteoblast specific factor	45.239	0	0
SULF1	sulfatase 1	22.135	0	0
CEACAM6	carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)	20.215	0	0
S100P	S100 calcium binding protein P	20.178	0	0
CEACAM5	carcinoembryonic antigen-related cell adhesion molecule 5	16.492	0	0
IGHV3-52	immunoglobulin heavy variable 3-52 (pseudogene)	16.002	3.38e-06	0.00139
IGKV2D-26	immunoglobulin kappa variable 2D-26	15.32	4.47e-06	0.001808
IGKV1D-33	immunoglobulin kappa variable 1D-33	15.19	5.17e-06	0.002053
VCAN	versican	13.152	0	0
SLC6A14	solute carrier family 6 (amino acid transporter), member 14	12.734	0	0
INHBA	inhibin beta A	12.479	0	0
COL1A2	collagen, type I, alpha 2	12.331	0	0
LCN2	lipocalin 2	12.008	0	0
IGKV1OR2-3	immunoglobulin kappa variable 1/ OR2-3 (pseudogene)	11.591	4.51e-07	0.000189
FN1	fibronectin 1	11.484	1.50e-13	1.08e-10
KYNU	kynureninase	10.716	0	0
CXCL5	chemokine (C-X-C motif) ligand 5	10.716	2.17e-10	1.09e-07
CTHRC1	collagen triple helix repeat containing 1	10.701	4.44e-16	4.45e-13
ITGB6	integrin beta 6	10.61	0	0
TFF1	trefoil factor 1	10.307	1.87e-09	8.97e-07
COL3A1	collagen, type III, alpha 1	10.249	0	0
SEMA3C	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	10.164	0	0

Validation based on TCGA and GTEx databases
To validate the results of this study, the GEPIA database was used to compare the expression levels of the identified enzyme-coding up-regulated genes between PDAC and normal pancreatic tissues. As shown in Figure 1 A and B, compared to normal pancreatic tissues, mRNA levels of kynureninase and sulfatase 1 were 7-fold and 3.5-fold higher in PDAC respectively ($p<0.05$). These data were consistent with the results above from the GEO data sets in the context of a statistically significant increase in the expressions of the two enzyme-coding genes in PDAC.

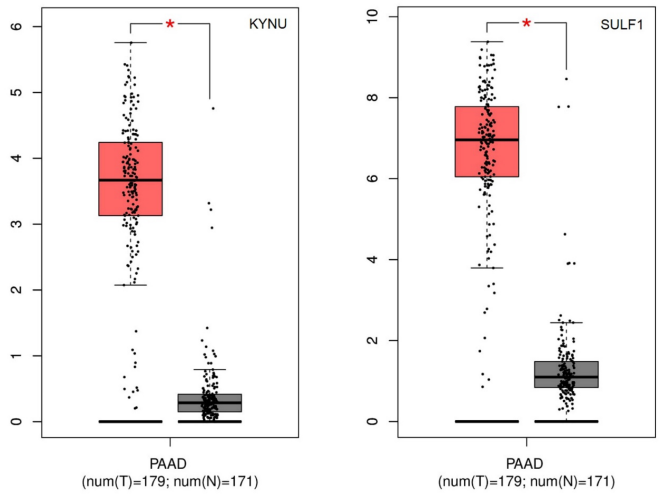

Figure 1. Expression levels of KYNU (A) and SULF1 (B) in cancer and normal tissues. PAAD: Pancreatic Adenocarcinoma, T: Tumor, N: Normal; *$p < 0.05$.

DISCUSSION

In the present study, an integrated transcriptomic meta-analysis was performed to suggest novel therapeutic targets and diagnostic biomarkers for PDAC. Based on the gene expression profiles of five GEO datasets, a total of 22 significantly over-expressed genes in PDAC were identified. Enzymes are preferred biomarkers for providing insight into the disease process by diagnosis, prognosis, and assessment of response therapy [19]. Besides, enzyme inhibitors constitute a significant portion of the druggable human proteome and the most important group of therapeutic agents that are in clinical use today [20, 21]. Thus, this study focuses on the identified up-regulated enzyme-coding genes in PDAC to suggest more effective therapeutic targets and diagnostic biomarkers for PDAC. Since the number of samples that met the stringent inclusion criteria of this study is small, only genes with at least ten-fold differential expression between PDAC and normal pancreatic tissues were considered as significant to increase reliability. Among the up-regulated genes identified in PDAC, two genes were found to encode enzymes. One of them was SULF1 (Sulfatase 1), which encodes an extracellular heparan sulfate endosulfatase that removes 6-O-sulfate groups from internal glucosamine residues in highly sulfated subdomains of heparin/HSPGs. The first study investigating the molecular function of SULF1 in pancreatic cancer was conducted in 2005 and reported a 22.5-fold increase in the mRNA level of SULF1 in pancreatic cancer compared to normal controls [22]. Later, two distinct studies showed that SULF1 protein level is significantly higher in pancreatic cancer tissues than in cancer-adjacent normal pancreatic tissues [23, 24]. In this study, a 22.31-fold increase in the mRNA level of SULF1 was detected in PDAC compared to normal pancreatic tissues, consistent with the results from TCGA and GTEx data, and previous reports. Overall findings suggest that sulfatase may have the potential to be an effective diagnostic biomarker for PDAC, which merits further investigation. However, only two studies have evaluated whether SULF1 inhibition has the potential to be an effective therapeutic strategy in pancreatic cancer. It has been reported that stable SULF1 expression resulted in reduced both anchorage-dependent and -independent cell growth; however, decreased FGF-2 mediated cell growth and invasion in SULF-1 negative Panc-1 pancreatic cancer cell line [22]. In 2007, Nawroth et al. reported that the expression of human extracellular sulfatases SULF1 and SULF2 enhances Wnt signaling in pancreatic cancer cells and contributes to the growth and tumorigenicity of these cells [25]. Thus, these divergent results above indicate a poor understanding of the complex molecular mechanism of SULF-1 in the pathogenesis of pancreatic cancer and underline the need for further studies in this field.

In the present study, the second significantly up-regulated enzyme-coding gene in PDAC was KYNU. It's product, kynureninase or L-kynurenine hydrolase is involved in the biosynthesis of NAD cofactors from tryptophan through the kynurenine pathway [26]. KYNU expression has been demonstrated to be up regulated in cases with pediatric acute lymphoblastic leukemia [27] and lung adenocarcinoma [28] before. The results of these studies also suggested that the high expression of KYNU correlates with poor prognosis in patients. Moreover, it has been reported that inhibition of KYNU may represent a novel therapeutic approach for the treatment of glioma [29]. However, there are also studies that have reported a decrease in KYNU expression in highly aggressive osteosarcoma cell lines [30] and a negative association between KYNU expression and tumor grade in breast cancer [31]. The altered KYNU expression and thereby its molecular function may be associated with metabolic reprogramming in cancer development which could be easily affected by the specific metabolic demands of different cancer types. Since high expression of KYNU may give rise to higher NAD+ levels, it may also provide a selective advantage to cancer cells by increasing glycolysis via glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH) that require NAD as a co-enzyme, and by fueling cancer cells [31]. However, the role of KYNU in pancreatic cancer and cancer metabolism is not yet known. Further studies addressing these issues may reveal therapeutic, diagnostic or prognostic values of altered KYNU expression in pancreatic cancer and its importance in pancreatic cancer metabolism.

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Illuminating the path to ISO 15189 accreditation: a view from the Republic of North Macedonia

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Abstract:
In the Republic of North Macedonia the work of the diagnostic medical laboratories is regulated by the Law of Health Care. There is an urgent need for better development of an evidence-based, scientific, and sustainable national strategy for the improvement of health laboratory service. Clear indicators of improvement have to be established. A key indicator should be the number of laboratories that have achieved and can maintain accreditation.
The Macedonian Society of Medical Biochemistry and Laboratory Medicine (MSMBLM) recommends that the quality system established meets the requirements of the International Standard for medical laboratories ('Medical laboratories: Requirements for quality and competence [EN ISO 15189:2012].
The accreditation of Macedonian medical laboratories is not mandatory; the decision for accreditation is voluntary. So far, nine medical laboratories have been accredited according the MKS EN ISO 15189:2013. Four of them are public sector laboratories.
The small number of accredited laboratories could be the result of the shortage of financial resources, poor government attention to laboratory service, the shortage of qualified personnel and/or the lack of a national laboratory policy.
The experiences of laboratory professionals from accredited laboratories, who have a high level of knowledge, skills, and competence, are crucially important to the process of developing a competent laboratory service within the national health system.
Key words: Accreditation, Quality Management System, ISO 15189, medical laboratories, biochemistry

ISO 15189 akreditasyonuna giden yolun aydınlatılması: Kuzey Makedonya Cumhuriyeti'nden görüşler

Özet
Kuzey Makedonya Cumhuriyeti'nde tıbbi laboratuvarların çalışmaları Sağlık Yasası ile düzenlenmiştir. Tıbbi laboratuvar hizmetinin iyileştirilmesi için kanıta dayalı, bilimsel ve sürdürülebilir bir ulusal stratejinin geliştirilmesine acil ihtiyaç vardır. Bu konuda açık iyileştirme göstergeleri tanımlanmalıdır. Temel bir gösterge, akreditasyon alan ve sürdüren laboratuvarların sayısı olmalıdır.
Makedonya Tıbbi Biyokimya ve Laboratuvar Tıbbı Derneği (MSMBLM), kurulan kalite sisteminin, tıp laboratuvarları için Uluslararası Standardın gerekliliklerini karşılamasını önermektedir ("Tıbbi laboratuvarlar: Kalite ve yeterlilik için şartlar" "[EN ISO 15189: 2012]).
Kuzey Makedonya'da tıp laboratuvarlarının akreditasyonu zorunlu değildir; akreditasyon kararı isteğe bağlıdır. Bugüne kadar MKS EN ISO 15189: 2013 uyarınca dokuz adet tıbbi laboratuvar akredite edilmiştir. Bunlardan dördü kamu sektörü laboratuvarıdır.
Kuzey Makedonya Cumhuriyeti'nde az sayıdaki akredite edilmiş laboratuvar bulunmasının nedenleri, finansal kaynakların yetersizliği, hükümetin laboratuvar hizmetine duyarsız kalması, kalifiye personel sıkıntısı ve / veya ulusal laboratuvar politikasının olmayışının sonucu olabilir.
Yeterli düzeyde bilgi, beceri ve yetkinliğe sahip olan akredite edilmiş laboratuvarlardaki laboratuvar uzmanlarının deneyimleri, ulusal sağlık sistemi içinde uzman bir laboratuvar hizmeti geliştirme süreci için çok önemlidir.

Anahtar kelimeler: Akreditasyon, Kalite Yönetim Sistemi, ISO 15189, tıbbi laboratuvarlar, biyokimya

INTRODUCTION

Laboratory medicine is a fundamental part of the modern healthcare system, and plays an important role in public health [1]. It is a very complex multidisciplinary diagnostic specialty with several interacting subdisciplines such as clinical biochemistry, hematology, immunology and others [2]. It depends on good knowledge of fundamental and applied sciences and the use of different technologies. Laboratory investigations are involved in every discipline of clinical medicine. Medical laboratories should ensure optimal test accuracy and precision, provide high quality laboratory test results, deliver test results in a timely manner and provide expert consultation to healthcare professionals [3]. However, legislators, clinicians, and patients are often unclear on the role of laboratory medicine as a specialty**.**

THE IMPLEMENTATION OF LABORATORY STANDARDS IN THE REPUBLIC OF NORTH MACEDONIA

In the Republic of North Macedonia the work of the diagnostic medical laboratories is regulated by the Law of Health Care [4]. Specific requirements for the laboratory service regarding capacities, equipment, health personnel and diagnostic tests are defined in the *Rule Book for the necessary space, equipment and personnel for founding, starting and performing healthcare procedures in a health care establishment* [5]. This document is insufficient; there is an urgent need for better development of an evidence-based, scientific, and sustainable national strategy for the improvement of health laboratory service. Clear indicators of improvement must be established. A key indicator should be the number of laboratories that have achieved, and can maintain accreditation.
Accreditation is formal recognition that a body or person is competent to carry out specific tasks. Meeting the International Standard means the laboratory meets the technical competence requirements and the management requirements to consistently deliver technically valid results.

Audit of *Management System* can be conducted by non-technical personnel. It includes assessment of management activities, such as:

- purchasing of consumable materials;
- management of calibration system;
- contracts / client interactions;
- document control and revision;
- training records.

Audit of *Technical Competence* must be conducted by someone familiar with test/methodology/performance of tests. The auditor assesses:

- sampling activities;
- preparation of reagents and/or samples;
- results / data analysis;
- performance of test.

The role of scientific societies

The Macedonian Society of Medical Biochemistry and Laboratory Medicine (MSMBLM) recommends that the quality system established meets the requirements of the EN ISO 15189:2012 "Medical laboratories: Requirements for quality and competence" (EN ISO 15189:2012) [6], which has been accepted as the fundamental standard for the accreditation of medical laboratories in European countries. EN ISO 15189 was developed as a baseline standard for the Quality Management System (QMS) in medical laboratories and is recognized as the connecting standard for all disciplines in laboratory medicine.
MSMBLM is a professional society of specialists in medical biochemistry. It is responsible for the translation of international guidelines into national guidelines. These guidelines have to be in agreement with the standard EN ISO 15189. For this purpose, cooperation between MSMBLM and the National Accreditation Body, Institute for Accreditation of the Republic of North Macedonia (IARM) [7] is essential. IARM, established in 2003, is a non-profit institution with a duty/task to act in the public interest. It is a subject to peer evaluation. In 2008 IARM has become a member of the International Laboratory Accreditation Cooperation (ILAC), which is an umbrella organization for regional accreditation cooperation. The acceptance of accredited laboratory data is facilitated through mutual recognition arrangements among accreditation bodies.
In Europe this is regulated by the European cooperation for Accreditation (EA) [8]. ISO 15189 is covered by the Health Care Committee (HCC) of EA, where, apart from almost all European Accreditation Bodies, European Federation of Clinical Chemistry and Laboratory Medicine and Medtech are represented [9-12].
The Law of accreditation was endorsed in 2009 (Official Journal of R.M. No. 120/2009). In 2013, the Standardization Institute of the Republic of North Macedonia accepted the standard as the Macedonian norm for quality assessment of medical laboratories (MKS EN ISO 15189:2013).
The accreditation of medical laboratories in Republic of North Macedonia is not mandatory; the decision for accreditation is voluntary. Accreditation is accessible to every client submitting an accreditation application to the National Accreditation Body. Assessment is performed every 4 years and surveillance is carried out annually. Flexible scope is not yet started but it is offered to accredited

laboratories that have already demonstrated that they have a verification/validation procedure that justifies the trust that comes with a flexible scope [13]. In 2013, the first medical biochemistry laboratory was accredited in our country. So far, nine medical laboratories of 240 registered laboratories have been accredited according to the MKS EN ISO 15189:2013. Four of them are public sector laboratories. Research Center for Genetic Engineering and Biotechnology "Georgi D. Efremov", which is a research unit of the Macedonian Academy of Sciences and Arts, and Diagnostic laboratories of the Institute of Pathology, Faculty of Medicine in Skopje are accredited according to MKS EN ISO 17025:2006.
The small number of accredited laboratories could be a result of the limited budget, especially for public medical laboratories, poor government attention to laboratory service, logistic and/or the lack of a national laboratory policy.
As the quality of laboratory service is a fundamental component of the healthcare system, the successful implementation of the accreditation process will bring a measurable quality improvement in laboratory service and, consequently, in the healthcare system [14-16]. Therefore, the Ministry of Health, professional associations, and other stakeholders should work together to make accreditation of medical laboratories a high priority and should undertake coordinated activities to integrate accreditation programs into national health policy and planning. Training by the Laboratory Quality Management system (LQMS) is a priority, with ISO 15189 accreditation as the ultimate goal [17].

Experience of implementing MK EN ISO 15189:2012 for accreditation of a university medical laboratory

Accreditation of a university laboratory is a difficult task, since teachers and teaching assistants have to coordinate different tasks, which makes the number of people involved in the process of accreditation quite variable. Implementation of a quality system on ISO 15189 and accreditation are completely achievable in this setting, in spite of the fact that this is a very demanding process. The experience of implementing a quality system on ISO 15189, and the accreditation of some tests for a university laboratory, is presented in the example of the Biochemical Analyses Laboratory (BAL), within the Institute of Medical and Experimental Biochemistry, the first public biochemical laboratory accredited according to MK EN ISO 15189:2013 in the Republic of North Macedonia.

Application for accreditation

Application should contain:

1. General data about the organization

1.1. Name and description of activities of the laboratory according to standard classification;
1.2. Legal identity of the organization;
1.3. Organizational structure of the laboratory and its position in the organization (with organizational scheme);
1.4. The names and position of key personnel with technical responsibility for testing and responsibility for quality assurance (head of laboratory, quality manager);
1.5. Personnel (technical staff);
1.6. Premises (locations where the tests are performed for the applied scope of accreditation);
1.7. When was the quality system according to standard *MKC* EN ISO 15189: 2013 implemented?

2. Scope of accreditation

Scope of accreditation identifies precisely the field of testing (classification according to IARM Regulation R 15). Scope of accreditation includes a listing of test methods, range of measurement, and biological material on which the testing is done.
The laboratory can claim accreditation only for the tests listed on the scope of accreditation.

3. Short description of the equipment

There has to be an appropriate equipment for realization of the respective laboratory tests. Equipment should be routinely calibrated and well-maintained**.**

The accreditation will be valid if:

1. Team members should have prior knowledge of the document

Document should be delivered to all staff members. They should be familiar with the technical and management requirements of the standard.

2. The accreditation team should be trained, competent and objective

The team is not going to be effective if it is composed solely of managers and leaders. Mutual trust and respect has to exist among the members of the team. The team has to promote safety and openness. If people feel that they are psychologically safe, they will be more open to share ideas, to say their opinion or to suggest new approaches. Dominance in the team should be avoided, but diversity is accepted, because diverse teams are much more productive. If there are opposite opinions in the team, people can overcome their way of thinking.
Every team member should be trained and competent to perform different tasks in the laboratory. Team members should be people who are at the front line, people who are closest to the process, people who are experts in each step of the testing process, people who perform some tasks every day and know to perform them in the best possible way. Not all team members need to be involved in all phases of the work, but they should be included when decisions related to their area are being made.

3. The laboratory should improve its performance continuously

Accreditation requires that a lab has a quality management system that focuses on continuous improvement. Improvement should not be done to people, but it should be created with people. When people come to work every day, they should think not only to do their work but to improve it. Continuous improvement is essential for success.
Improvement should be a part of the culture of the organization and a natural part of how people perform their jobs.

Key steps in the process of accreditation

1. Development of a Quality Manual

The Quality manual is the key document describing the overall Quality Management System. It should provide an understanding of the laboratory management and organizational structure; it describes roles and responsibilities of Quality Manager and Laboratory Director; it gives framework for design and implementation of the quality management system in the lab.
All staff members have to be familiar with the content and application of the quality manual and the referenced documents.

2. Development of process control

In terms of process control, preparation of standard operational procedures (SOPs) and working instructions are necessary in the initial phase.
SOPs are documents with step-by-step written instructions for each procedure performed in the laboratory. The purpose of these documents is to make sure that all procedures are performed consistently (correctly and always in the same manner) by everyone in the laboratory. A well-known rule in the process of SOP creation is 'write what you are doing and do what you're writing'.

3. Development of a management system for documented information

Every laboratory should have a control over the documented information. All relevant documents, records and policies have to be managed under controlled conditions and have to be reviewed and approved by authorized personnel prior to use. A written system of document preparation with declared responsibility for the documents. Structured and organized control of documents allows the lab to function more efficiently. Managing and controlling of documents is an overwhelming process, but the time and effort put into improving the laboratory performance are well worth it.

4. Internal quality control (IQC)

IQC is a segment of the quality management system used to ensure the accuracy of the results. It involves the in-house procedures to ensure day-to-day consistency of an analytical (examination) process. All personnel should be trained for

implementation of IQC. If QC results are not acceptable, laboratory should not report patient results.

5. External Quality Assessment (EQA)

EQASs are designed for the objective assessment of the laboratory's performance using an external agency or facility. All laboratories should be enrolled in EQAS program, a process in which a laboratory receives an unknown sample to test and report the findings back to the EQAS provider. EQA should not be punitive. It should be used as a tool for improvement of laboratory performance. EQA programs will assist laboratories in improving analytical quality, inter-laboratory agreement, identify potential equipment or reagent failures, and identify any training deficiencies. Some EQA programs are voluntary while others are obligatory, either required by an accrediting body or by law. For laboratories that are accredited, or that plan to seek accreditation, EQA participation is usually required.

6. Sample storage

Every laboratory should have written policies for types of samples that can be stored, correct sample labeling, location of stored samples, retention time, ambiental conditions for storage, maintenance and disposal of samples.
The laboratory should have standard procedures for safety issues like disposal of all laboratory waste and patient samples in accordance with national regulations for disposal of medical waste.

7. Reporting of results

Results should be reliable and accurate and reported in a timely manner.
Every laboratory should have a procedure to ensure correctness of transcription of laboratory results.
Each laboratory should have written procedure for reporting of critical results.
Comments on sample suitability with respect to acceptance/rejection criteria or sample quality that might compromise test results should be included.
The content of the report should be according to the requirement of the standard EN ISO:15189:20112 (section 5.8.3).

8. Laboratory Information System (LIS)

An integrated Laboratory Information system (LIS) is crucial for the efficient management of work processes, quality control and financial planning. It is hard to imagine a modern diagnostic laboratory functioning without the use of a LIS. A good information management system will ensure that all data, including the final report is well managed; LIS should ensure the accessibility, accuracy, timeliness and security of data, as well as confidentiality and privacy of patient information.

Critical elements for improvement of the laboratory quality management system

In the 10-year-period as an accredited laboratory, we have identified critical elements for improvement of the laboratory quality management system.
Communication
Communication is essential for quality. Each team member should be informed what was done in the past, what is in progress and which are future plans. Dissemination of information within the laboratory and between the laboratory and its customers is crucial for effective fulfillment of requirements and needs of customers.
Cause and effect diagram (Fishbone diagram)
One of the basic tools of quality improvement is the Fishbone diagram which identifies the possible causes of errors, called failure modes, and their effects. *Failure modes effect analysis (FMEA)* allows identification of the effects (criticality, severity and probability of occurrence).
Root Cause analysis is a system of problem solving methods aimed at identifying the root cause of problems or incidents. By directing corrective measures at root causes, it is hoped that the likelihood of problem recurrence will be minimized.
Participation in EQAS
EQA is one of the critical elements for improvement of the laboratory quality management system because it represents a measure of laboratory performance.
A laboratory must develop a plan to demonstrate coverage of the test methods on its scope of accreditation over a 4-year-period. It is mandatory to demonstrate successful participation in EQAS. Acceptance criteria is +/- 2SD. Corrective action has to be taken for any outlying results.
As accredited laboratory, BAL has an obligation to fulfill two documents: plan for participation in EQAS/PT (document OB 05-18-2) and review of participation (document OB 05-18) and to present them to the lead assessor and technical assessor on annual basis during the on-site assessment of the laboratory.
Some benefits of EQAS participation include:
- Discovering of sources of error,
- Systematic errors,
- Demonstration of effectiveness of changes,
- Common understanding of method differences,
- Discovery of method sensitivities,
- Comparison of laboratory's internal methods against standard methods,
- It can be a source of method validation for new measurement techniques, methods, etc.
- EQAS also can lead to method/standard improvement.

Corrective and Preventive actions
Accreditation requires that a lab have a quality management system that focuses on continuous improvement. A critical part of that management system is identifying when the system doesn't conform to the requirements and taking action to correct the problem and eliminate the root cause to avoid recurrence of the problem.
Implemented corrective and preventive actions need to be periodically monitored to ensure that they were effective and resolve the problem or potential problem.

Overall benefits of accreditation

• Increased confidence in data;
• Reduced uncertainties associated with decisions;
• Issues with methods, personnel, and equipment are identified and resolved more quickly;
• Customer satisfaction is improved;
• Business opportunities may increase.

Conclusions and future directions

The globalization of markets and migration of health professionals requires improving the laboratory diagnostic process. A quality laboratory system is the foundation of a strong national health system [18-21]. Laboratory workforce, infrastructure, and quality management system are vital for the delivery of quality laboratory services. Coordination with the Ministries of Education and Health is essential for maintaining standards of education and levels of knowledge. The competency of laboratory professionals has to be maintained through mandatory participation in continuous medical education (CME). Appropriate laboratory facilities, infrastructure, and equipment for each laboratory tier level are essential to enable safely and efficient performance. Strong programs supporting quality assurance, quality control, and quality improvement should exist. They are fundamental for the establishment, maintenance and improvement of laboratory quality systems. SOPs must be well-written, understood, and implemented; laboratory personnel should routinely perform IQCs; and laboratories must be required to participate in EQA or proficiency testing (PT) programmes.

For Government, Ministry of Health, professional association(s) and stakeholders, accreditation of medical laboratories according to IS 15189:2012 should be a high priority. They should act together and undertake coordinated efforts to integrate accreditation programs into national health policy, planning, and health development programmes.

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Induction of APAF-1 and TRAIL by bilberry tea in HCT-116 colon cancer cell line
[Yabanmersini çayı ile HCT-116 kolon kanseri hücre hattında APAF-1 ve TRAIL'in uyarılması]

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ABSTRACT

Objectives: In this study, we aimed to determine the effect of bilberry tea samples on the markers of the intrinsic and extrinsic pathways of apoptosis in the HCT-116 colon cancer cell line.

Materials and Methods: Bilberry tea in different infusions and boiling periods (1 min, 3 min, 5 min, 10 min) were prepared and phenolic levels were determined by LC MS / MS technique. The highest phenolic content was determined in tea samples of dried shredded fruit for 5 min boiling, so this product was chosen for *in vitro* study. Cytotoxicity and viability tests were performed by adding WST-8 solution. Intrinsic and extrinsic pathways of apoptosis were assessed by determining the TNF-related apoptosis-inducing ligand (TRAIL), Apoptotic Protease Activating Factor-1 (APAF-1), Cytochrome-c, Caspase -3,-8,-9 levels in HCT-116 colon cancer cell line.

Results: Cytotoxicity studies in cell culture were conducted using 50-10 µg/ml of bilberry tea samples which was prepared at a concentration of 5 g/10 ml. The levels of Caspase 3, APAF-1, TRAIL and Cytochrome-c were significantly higher in bilberry added cell culture than the control cells. Other markers (caspase -8, -9 levels) did not show any significant change compared to control cells.

Conclusions: It is concluded that bilberries induced TRAIL, APAF-1, Caspase-3, Cytochrome-c and consequently induced both intrinsic and extrinsic pathways of apoptosis.

Keywords: APAF-1, TRAIL, bilberry, HCT-116, colon cancer

ÖZET

Amaç: Bu çalışmada, yaban mersini çayı örneklerinin HCT-116 kolon kanseri hücresindeki apoptozun iç ve dış yolaklarında apoptoz belirteçleri üzerindeki etkisinin belirlenmesi amaçlandı.

Malzeme ve yöntemler: Yabanmersini çayı farklı infüzyonlarda ve kaynama sürelerinde (1 dak, 3 dak, 5 dak, 10 dak) hazırlandı ve fenolik seviyeler LC MS / MS tekniği ile belirlendi. En yüksek fenolik içerik, 5 dakika kaynama için kuru paralanmış meyvelerin çay örneklerinde belirlendi, bu yüzden bu ürün in vitro çalışma için seçildi. Sitotoksisite ve canlılık testleri WST-8 çözeltisi ilave edilerek yapıldı. Apoptozun iç ve dış yolakları, HCT-116 kolon kanseri hücre hattında TNF-related apoptosis-inducing ligand (TRAIL), Apoptotic Protease Activating Factor-1 (APAF-1), Sitokrom-c, Kaspaz -3, -8, -9 seviyelerinin belirlenmesiyle değerlendirildi.

Bulgular: Hücre kültüründe sitotoksisite çalışmaları, 5 g / 10 ml konsantrasyon da hazırlanan 50-10 ug / ml yabanmersini çay numunesi kullanılarak gerçekleştirildi. Yabanmersini çay örnekleri eklenmiş hücre kültüründe kaspaz-3, APAF-1, TRAIL ve Sitokrom-c seviyeleri kontrol hücrelerine göre anlamlı derecede yüksek bulundu. Diğer belirteçler (kaspaz -8, -9 seviyeleri) kontrol hücrelerine kıyasla anlamlı bir değişiklik göstermedi.

Sonuç: Yabanmersininin TRAIL, APAF-1, kaspaz-3, sitokrom-c'yi indüklediği ve sonuç olarak apoptozun hem iç hem de dış yolaklarını indüklediği sonucuna varılmıştır.

Anahtar Sözcükler: APAF-1, TRAIL, yaban mersini, HCT-116, kolon kanseri

Introduction

Cancer; is a pathological condition caused by disruption of the balance between cell proliferation and cell death due to excessive cell proliferation or decreased apoptosis. It has been reported that suppressed or decreased apoptosis plays an important role in cancer development (1). According to data from the American Cancer Society, 12.5% of annual deaths worldwide are caused by cancer and approximately 8,8 million people die annually from cancer (2). The probability of lifetime cancer diagnosis is 39.7% for men and 37.6% for women. The most common cancers to be diagnosed in men are prostate, lung and colorectal cancers, which constitute 42% of all cases. In women, the most common cancers to be diagnosed are breast, lung and colorectal cancers, representing half of all cases (3).

Chemotherapy is one of the commonly used methods for cancer treatment. Two target mechanisms come into prominence in preventing the proliferation of cancer cells with chemotherapeutic drugs. These are inhibition of cell growth or activation of apoptosis. Stopping the cell cycle is an effective strategy in eliminating cancer cells. Apoptosis is regulated by the factors in intrinsic and extrinsic signaling pathways (4).

In recent years, the use of natural sources as anticancer agents has become a popular research area, because of the resistance to anticancer drugs and cytotoxic effect of these anticancer agents against normal cells in long-term usage (5). Plants have been used for centuries to overcome human and animal diseases. Today, approximately 50% of the modern drugs used to suppress the proliferation of cancer cells in the clinic are obtained from natural products (6). According to the estimates of the World Health Organization, approximately 80% of the people living in developed countries use traditional treatment to solve their primary health problems (5). Therefore, natural products are considered as potential pharmaceutical raw materials (1).

The aim of this study was to investigate the effect of bilberry tea sample on the molecules of apoptosis pathways in HCT-116 colon cancer cell line.

Material and method

Materials

McCoy's 5A, Fetal Bovine Serum, Penicilin/Streptomycin, L-Glutamin were purchased from Capricorn Scientific (Ebsdorfergrud, Germany). All of the phenolic compound standards, Dimethyl sulphoxide (DMSO) and Trypan Blue were purchased from Sigma (USA). WST-8 Cell Proliferation Assay Kit were purchased from Cayman Chemical (Michigan, USA). Human (TRAIL) and Caspase (-3,-8-9 ELISA Kits were purchased from Genosis (USA). Human Apoptotic Protease Activating Factor-1 (APAF-1) ELISA Kit was purchased from Biosens (USA).

Bilberry Tea Preparation

Bilberries were harvested from Menderes region near to Izmir. Hand harvested fruits were destemmed and calibrated on the basis of equal sizes. Samples were stored at -20ºC before analyses. As study material, different forms of bilberry fruit and leaves were used: dried leaves, dried fruit, dried shredded raw fruit, frozen raw fruit, seedless fruit and fruit seeds. Dried leaves (DL) were separated manually from aerial parts in laboratory and dried at 60^0 C for 15 min in tray dryer. Processed leaves were finely ground with small home blender (Joyce home electrical accessory Co. Ltd). Dried fruits (DF) were obtained by similar drying processing procedure with different conditions: 40^0C for 60 min. Dried shredded fruits (DSF) were processed in similar way to dried fruit and finely ground with small home blender (Joyce home electrical accessory Co. Ltd). Frozen fruits (FF) were obtained by freezing the fruits at -15^0 C for 24h. Seedless fruits (SF) were prepared by manual separation of seeds in laboratory. Fruit seeds (FS) were also obtained and used.

Tea processing was done by using two types of extraction procedures: infusion and boiling. Infusion was done by keeping the obtained products in previously boiled water 100^0 C/ 5 min for different times (1 min, 3 min, 5 min, 10 min). Boiling was carried out by keeping the obtained products in continuously boiling water (100^0 C)for different times (1 min, 3 min, 5 min, 10 min).

Determination of the amount of phenolic compounds by LC MS / MS Analysis

Quantitative analysis of the components was performed using the external standard method. The analysis were performed on Waters Xevo TQD system (Waters) containing automatic sample injection and UHPLC. Acquity UPLC BEH C18 (50 x 2.1 mm, 1.7 µm) column was used for the separation. The spectral range covered was from 190 to 360 nm. Column temperature of 45 ˚C and injection volume of 5 µL were selected. Based on the molecular spectrum of the standards, two wavelengths were used. The flow rate was 0.3 mL/min and the UV scan was carried out in the range of 254-280 nm. The optimum pH value for the separation efficiency was chosen between 2-3. The mobile phase was designated to be methanol + 0.1% formic acid (FA) and water + 0.1% formic

acid. A linear gradient defined was used for elution.

Cell culture

HCT-116 human colon cancer cell line was purchased from American Type Culture Collection (ATCC) and were multiplied in McCoy's 5A medium supplemented with 10% fetal calf serum (FBS), 100 unit/ml penisilin, and 100 µg/ml streptomycine and incubated at 37°C in a humid environment containing 5% CO_2.

Determination of Apoptosis in Cell Culture

The highest phenolic content was determined in tea samples of DSF for 5 min boiling, so this product was chosen for *in vitro* study. After the HCT-116 colon cancer cells were plated in 6-well plates at 1 million/ mL, the tea sample was applied and the cells were collected following the incubation period of 48 hours, centrifuged for 20 min at 2000-3000 rpm. Later, they were centrifuged at 2000-3000 rpm for 20 minutes again making icing-deicing application three times. Human TRAIL, Human APAF-1) Cytochrome-C, Caspase-3, Caspase-8, Caspase-9 were determined with commercially available ELISA kits.

Results

The main phenolic contents of tea samples are presented in Table 1 and Table 2.

Table 1: LC MS/MS results of phenolic compounds (ng/ml) of different samples with different brewing times

Brewing	Caffeic acid	Kaempherol	Ferulic acid	Quercetin	Vanilic acid	Ellagic acid	Delphinin	Pelargonin
Dry Leaf 1 min	11,80	12,32	27,35	141,48	397,54	25,13	18,08	40,71
Dry Leaf 3 min	7,57	23,59	59,88	271,63	346,92	41,97	73,65	35,40
Dry Leaf 5 min	7,95	44,27	145,33	253,64	1079,18	31,85	56,88	184,28
Dry Leaf 10 min	6,52	20,76	41,06	165,03	225,82	30,11	45,88	13,38
Dry Fruit 1 min	8,92	80,10	218,04	732,54	1475,20	82,49	152,25	185,34
Dry Fruit 3 min	5,91	25,99	77,37	155,21	183,79	17,24	40,19	30,98
Dry Fruit 5 min	6,75	63,11	261,18	742,14	581,75	68,17	166,12	237,17
Dry Fruit 10 min	6,37	16,04	55,35	253,26	158,40	33,81	59,92	24,34
Dry shredded raw fruit 1 min	5,65	527,84	344,13	7087,67	128,29	689,70	1551,26	176,98
Dry shredded raw fruit 3 min	6,09	432,75	359,15	5222,89	286,45	511,49	1206,45	85,61
Dry shredded raw fruit 5 min	7,01	482,17	457,65	6091,25	537,68	567,81	1373,23	169,98
Dry shredded raw fruit 10 min	5,81	430,24	267,54	5341,57	314,43	501,16	1126,68	41,69
Frozen Raw Fruit 1 min	6,31	6,23	38,85	135,27	284,45	14,55	19,94	25,74
Frozen Raw Fruit 3 min	9,71	30,08	146,20	180,71	424,58	22,97	54,40	120,20
Frozen Raw Fruit 5 min	6,22	22,75	229,22	155,37	790,04	20,08	56,35	224,57
Frozen Raw Fruit 10 min	6,26	24,51	90,18	268,71	368,46	35,09	88,48	45,69
Seedless Raw Fruit 1 min	6,19	307,94	255,05	3126,47	473,41	314,31	738,73	32,30
Seedless Raw Fruit 3 min	8,93	376,44	352,40	3818,71	471,71	375,09	951,53	74,56
Seedless Raw Fruit 5 min	6,84	424,30	350,05	4471,08	615,41	423,11	1043,69	60,50
Seedless Raw Fruit 10 min	6,76	686,86	487,14	6751,40	635,44	628,97	1686,82	160,43
Seed 1 min	6,88	47,66	149,64	865,45	573,72	100,78	211,40	54,72
Seed 3 min	7,09	67,08	149,82	1196,82	361,10	135,89	235,47	70,07
Seed 5 min	6,66	76,59	145,41	1246,56	450,74	150,19	244,98	65,91
Seed 10 min	6,52	118,13	133,37	1697,29	513,23	192,09	361,44	40,07

Table 2: LC MS/MS results of phenolic compounds (ng/ml) of different samples with different boiling times

Boiling	Caffeic acid	Kaempherol	Ferulic acid	Quercetin	Vanilic acid	Ellagic acid	Delphinin	Pelargonin
Dry Leaf 1 min	6,47	38,73	101,77	451,77	901,78	62,54	89,04	41,77
Dry Leaf 3 min	13,73	75,25	125,21	734,96	1213,75	97,03	182,43	45,40
Dry Leaf 5 min	6,02	35,30	50,42	433,42	301,02	52,62	99,26	20,54
Dry Leaf 10 min	10,85	66,94	169,24	770,76	1023,84	98,27	158,73	78,81
Dry Fruit 1 min	5,33	59,67	127,77	522,91	688,03	46,51	115,77	25,28
Dry Fruit 3 min	5,72	350,03	225,26	3256,67	461,12	308,18	748,29	50,41
Dry Fruit 5 min	4,43	382,02	252,89	3323,46	458,95	324,22	726,24	69,71
Dry Fruit 10 min	5,69	880,81	283,16	7582,45	634,29	703,52	1678,75	49,77
Dry shredded raw fruit 1 min	6,18	524,97	269,41	6087,00	246,92	576,74	1400,81	13,50
Dry shredded raw fruit 3 min	6,01	1164,79	387,65	11405,38	365,27	1064,93	2643,94	72,69
Dry shredded raw fruit 5 min	6,30	1305,19	496,37	12366,48	426,60	1155,62	2891,57	140,52
Dry shredded raw fruit 10 min	6,77	83,28	45,18	1177,79	51,91	137,49	234,83	8,83
Frozen Raw Fruit 1 min	7,57	128,18	163,74	1301,13	607,13	157,26	425,35	135,81
Frozen Raw Fruit 3 min	7,51	184,72	196,88	1692,92	499,54	193,74	448,80	74,97
Frozen Raw Fruit 5 min	8,12	296,59	284,84	2773,51	723,99	279,90	708,98	125,16
Frozen Raw Fruit 10 min	7,05	180,74	154,12	1634,45	479,02	172,85	412,49	42,74
Seedless Raw Fruit 1 min	7,35	546,77	386,58	4718,60	548,74	500,76	1141,49	106,55
Seedless Raw Fruit 3 min	6,41	936,90	395,29	7957,13	623,00	787,05	2129,66	65,90
Seedless Raw Fruit 5 min	8,47	1000,08	459,06	8336,39	681,25	799,32	2020,68	87,23
Seedless Raw Fruit 10 min	8,07	1044,13	498,43	8399,59	827,27	823,19	2152,48	126,79
Seed 1 min	6,80	115,72	185,85	1829,68	505,82	214,69	407,78	40,71
Seed 3 min	6,28	196,87	216,22	3161,61	445,67	340,53	698,16	84,59
Seed 5 min	6,18	240,38	186,29	3392,69	332,00	371,69	758,12	39,90
Seed 10 min	5,52	346,72	199,28	4427,19	236,86	442,46	992,95	78,81

When the above tables were examined, the infusion method with the highest phenolic molecule content was determined as 5 minutes boiling of DSF. Therefore 5 min boiling DSF sample was used for cell culture studies. The IC50 values of bilberry extract was shown in Figure 1.

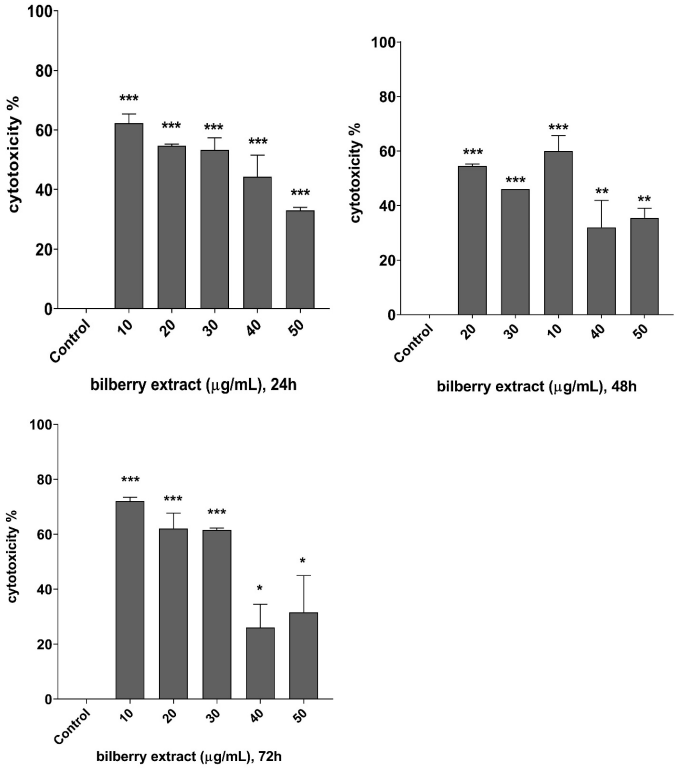

Figure 1: IC_{50} concentrations for bilberry extract in HCT-116 colon cancer cell culture. It was calculated for 3 different periods: (0–24 h, 24–48 h and 48–72 h). The IC50 values were 101,680 µg/mL, 80.676 µg/mLand 106,180 µg/mL in 24th, 48th and 72th hours, respectively. As shown in Figure 1, the bilberry extract inhibited HCT-116 colon cancer cells proliferation in time-dependent manner with the highest IC50 (50% inhibitory concentration) value of 80.676 µg/ml after 48 h treatment.

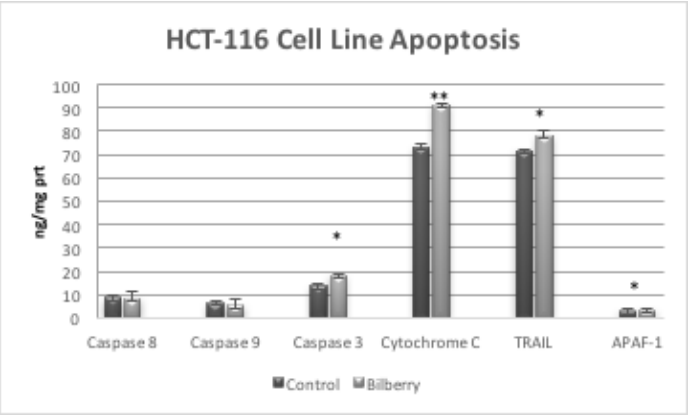

Figure 2: Effect of bilbery extract on apoptotic biomarkers in HCT-116 cell line**;** the levels of Caspase (-8, -9 -3), Cytochrome-c, TRAIL and APAF-1.

Cytotoxicity studies in cell culture were conducted using 50-10 µg/ml of bilberry tea samples which was prepared at a concentration of 5 g/10 ml. As it can be seen from the Figure.2. The levels of APAF-1, TRAIL, Cytochrome-c and Caspase 3 were significantly higher in bilberry added cell culture than the control cells. Other markers (Caspase -8, -9 levels) did not show any significant change compared to control cells.

DISCUSSION

It is proposed that many compounds derived from natural plants act like chemical inhibitors in human cancer treatments, thus may have chemotherapeutic activity (7, 8, 9). Some studies have shown that bilberries manifest antioxidant (12), anti-inflammatory (13), antibacterial (10, 14], hypoglycemic (15), antifungal (16), and antimutagenic (17) effects. In this study, the apoptotic effect of bilberry tea

on HCT-116 colon cancer cell line was assessed.

The studies with natural compunds have shown that the different solvents, and the methods used to dissolve the plant or the fruit affects the study results related to active compounds in the plant or fruit studied. In the studies of Yildiz et al. (2011) related to Vaccinium myrtillus L., the structure of the biological active components of Vaccinium myrtillus L. were clarified, and, the collected fruits were stored at -18˚C for 1 week, and then dried at 40˚C and pulverized. Dried fruits were extracted with methanol, ethanol, water and ethyl acetate, and left to stand in the stirrers with heater for 24 hours. The analyzes were performed by RC-HPLC. Researchers investigating the amount of phenolic compounds have found that the pehnolic content of plants show high variability due to the factors such as location, growing conditions, soil properties, irrigation, temperature and sunbathing. Previously, caffeic acid (6.29 ± 3.55 µg/g), epicatechin (8.35 ± 5.04 µg/g), and myricetin (4.16 ± 1.81 µg/g), were found as the highest phenolic molecules in bilberry extracts (2.10 ± 0.64 µg/g) (19). The most promising anticarcinogenic agents in plants are these phenolic compounds, which are abundantly present in Bilberries (Vaccinium myrtillus) (20).

In these studies, the bilberry fruits were extracted in the different types of solvents (water, ethanol and ethyl acetate), and their chemical compositions were identified using HPLC - DAD and HPLC-MS/MS method and the effects on antioxidant system were investigated using in-vitro analysis. In the result of these experimental studies, antiradical and antioxidant effects were found to be higher in ethanol, and ethyl acetate extracts, as well as high amounts of chemical compounds in these extracts.

The healthiest way of using these plant extracts is to solve them in water. therefore, boiling and brewing were used in our study. Since the studies have shown that brewing and boiling duration are effective in separating phenolic compounds from plants, we determined the phenolic content of bilberry fruits in different extraction times. Our data showed that the amount of obtained phenolic molecules vary depends on duration and extraction methods. Generally, the extracted amount was higher in boiling methods compared to those of brewing method and the highest phenolic molecule was quercetin both in two methods.

In the study of León-González (2018), Anthocyanin- rich bilberry extract "Antho 50" induced apoptosis and down-regulated the expression levels of different epigenetic proteins in human leukemia Jurkat cells. They observed that bilberry extract induced a redox-sensitive caspase-3- mediated apoptosis in chronic lymphocytic leukemia cells (21). In consistent with the mentioned study, the bilberry tea which include of kaempherol, quercetin, myricetine and delphinin in high concentration induce caspase-3 levels.

In our study, the extrinsic pathway in the HCT-116 colon cancer cell line is stimulated, which was confirmed with an increase in the TRAIL levels.

It has been suggested that ethanol extract of bilberry, rich in anthocyanins, inhibited the growth of HL60 human leukemia cells through the induction of apoptosis. When anthocyanidins and the anthocyanins isolated from bilberry extract were compared, the anthocyanins, mainly delphinidin- and malvidin-glycosides caused apoptosis in the HL60 cells. The bilberry ethanol extract and certain anthocyanins inhibited the growth of HCT116 human colon carcinoma cells (22). In accordance with this study, bilberry tea sample having the high anthocyanins induced the apoptosis in our study.

In conclusion, the antioxidant and anticancer effect of natural products is related to its main phenolic content which are extracted by ethanol, water, methanol etc. The amount of extracted phenolic molecules might change due to extraction methods and extraction duration. Bilberry tea may contribute to maintain plasma levels of anthocyanins, which possess antioxidant activity and inhibitory effects on cancer cell growth via inducing apoptosis. Therefore, Bilberry tea might be suggested as a functional food for cancer treatment however further in vitro researchs need to clarify the required dose and effect of billberry tea.

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Author Contributions: EYS designed the study&prepared the study protocol. BD obtained literature data. HKY prepared the tea samples BD&HM performed LC MS/MS analysis. BD, NSG, LO designed the experiments&performed cell culture studies. ES, BD analysed the data.

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Development and validation of a biosensor for measurement of serum hypoxia-inducible factor-1
[Serum hipoksi ile indüklenebilir faktör-1'in ölçülmesi **için bir biyosensör geliştirilmesi ve validasyonu]**

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Abstract

Objectives: Normal oxygen delivery is essential for survival. Hypoxia, which is a common feature of various pathological conditions, ranging from cancer to inflammatory diseases, occurs when normal oxygen delivery is altered by an imbalance between cellular oxygen demand and tissue oxygen supply. Among the intricate mechanisms organisms have developed to maintain oxygen homeostasis, a family of hypoxia-inducible transcription factors (HIFs), are found to be the main regulator adaptive cellular response to hypoxia. Although ELISA can be used for its measurement, the lability of the protein and length of the analysis (>5 hours) pose limitations. Thus, our aim is to develop an electrochemical impedance spectroscopy (EIS) based biosensor system for quick and reliable measurement of HIF-1α in serum.

Materials and Methods: HIF-1alpha antibodies have been used as a biorecognition receptor. For immobilization, the electrode was first modified with albumin, followed by PAMAM. The new biosensor was compared with the conventional ELISA method.

Results: Based on the chronoimpedance data, total analysis time for EIS was chosen as 15 minutes. Calibration curve was constructed by locating electron transfer resistance on y-axis and HIF1 concentration on x-axis, between 50-1000 pg/mL. LOD and LOQ of the biosensor were calculated as 14.45 pg/mL and 43.8 pg/mL, respectively. The new biosensor showed very good correlation when compared with the conventional ELISA method (R2= 0.99649).

Conclusion: We developed and analytically validated a biosensor system to measure HIF-1α in serum. This new biosensor promises more timely and accurate measurements in determining the tissue oxygenation in patients who have hypoxia related conditions such as diabetic foot.

Keywords: hypoxia inducible factor 1 alpha, biosensor, impedance, PAMAM

Öz**et**

Amaç: Normal oksijen iletimi hayatta kalmak için esastır. Kanserden inflamatuvar hastalıklara kadar çeşitli patolojik durumların ortak bir özelliği olan hipoksi, normal oksijen iletiminin hücresel oksijen talebi ile doku oksijen beslenmesi arasındaki dengesizlik sonucu etkilenmesiyle meydana gelir. Organizmaların oksijen homeostazını korumak için geliştirdiği kompleks mekanizmalar arasında, hipoksiye karşı ana düzenleyici hücresel uyum yanıtının hipoksi ile indüklenebilir transkripsiyon faktörleri ailesi (HIF) olduğu bulunmuştur. Her ne kadar ELISA ölçümü bu belirteç için kullanılabilse de, proteinin labil doğası ve analiz süresinin uzunluğu (> 5 saat) bu ölçüme sınırlamalar getirir. Bu nedenle amacımız serumda HIF-1α'nın hızlı ve güvenilir ölçümü için elektrokimyasal empedans spektroskopisi (EIS) bazlı biyosensör sistemi geliştirmektir.

Gereç ve Yöntem: HIF-1 alfa antikorları, biyotanıma reseptörü olarak kullanılmıştır. İmmobilizasyon için elektrot önce albümin, ardından PAMAM ile modifiye edilmiş ve geliştirilen yeni biyosensör, geleneksel ELISA yöntemiyle karşılaştırılmıştır.

Bulgular: Kronoimpedans verilerine dayanarak, EIS için toplam analiz süresi 15 dakika olarak seçilmiştir. Kalibrasyon eğrisi, y-ekseni üzerindeki elektron transfer direnci, x ekseni üzerinde HIF-1 konsantrasyonu 50-1000 pg/mL arasında yerleştirilerek yapılmıştır. Biyosensörün LOD ve LOQ değerleri sırasıyla 14,45 pg/mL ve 43,8 pg/mL olarak hesaplanmıştır. Yeni biyosensör, geleneksel ELISA yöntemiyle karşılaştırıldığında çok iyi bir korelasyon göstermiştir (R^2 = 0,99649).

Sonuç: Bu çalışmayla, serumda HIF-la'yı ölçmek için bir biyosensör sistemi geliştirilmiş ve sistemin etkinliği analitik olarak doğrulanmıştır. Bu yeni biyosensör, diyabetik ayak gibi hipoksi ile ilişkili rahatsızlıkları bulunan hastalarda doku oksijenasyonunun belirlenmesinde daha kısa zamanda ve daha doğru ölçümler vermeyi vaat etmektedir.

Anahtar Sözcükler: hipoksi indüklenebilir faktör 1 alfa, biyosensör, empedans, PAMAM

Introduction

Normal oxygen delivery is essential for survival. Recently, 2019 the Nobel Prize in Medicine (or Physiology) was given to three scientists for their studies on adaptation of the organism in hypoxic conditions [1]. There are different biomarkers that can show the level of hypoxic conditions and one of them is hypoxia-inducible factor-1 (HIF-1). HIF-1 is a transcription factor that plays a key role in the development of adaptation response to hypoxia. The molecule consists of the subunits HIF-1α and HIF-1β [2]. HIF-1α is the main regulator of oxygen homeostasis and adaptive cellular response to hypoxia to regulate cell survival [3]development, physiology, and pathobiology. The hypoxia-inducible factors (HIFs. It is activated by a decrease in oxygen concentration of the cell and targets many genes in various pathways; erythropoiesis, iron metabolism, angiogenesis, glucose metabolism, vascular tonus, matrix metabolism, cell survival, proliferation and apoptosis. Studies have shown that HIF-1α dysfunction occurs in the hyperglycemic environment in diabetic individuals as a result of disruption of transactivation. The increased level of reactive oxygen radicals in the hypoxic environment inhibits HIF-1α gene expression. Interestingly, some HIF-1α polymorphisms were found to be significantly higher in diabetic foot ulcer patients when compared to that of healthy controls, so these mutations can be important in the pathogenesis of diabetic foot wound under hypoxic conditions [4].
The conventional method for HIF-1α analysis is the ELISA which has some limitations due to length of the analysis (>5 hours) and the lability of the protein. Besides matrix effects like hemolysis, hyperlipidemia can also alter the measurement accuracy in the ELISA system. Thus, our aim was to develop an electrochemical impedance spectroscopy (EIS) based biosensor system for quick and reliable measurement of HIF-1α in serum.
Impedimetric biosensors are developed by immobilization of a receptor; an antibody, DNA or protein molecule, which show affinity only to the analyte molecule. The signals resulting from the interaction between this biological sensor and the analyte molecule are transmitted by the physicochemical transducer to the analysis system and measurement can be performed by analyzing the concentration-dependent response of these signals obtained through EIS [5]Biosensor technology has provided a number of benefits to detect both biological and chemical molecules. Abiosensor is a promising device, which is combination of sensitivity of electrochemistry and specificity of biological recognition, enables to detect any kind of molecules in a short time with selectively and sensitively. Likewise many analytical methods, it has also limitations, such as high oxidation potentials lead to detection of non-target molecules, furthermore non-electroactive species cannot show electroactive signal for measurement or some biomolecules cannot be transformed by enzymes, even if they can be transformed, they require secondary molecules such as mediators, coenzymes or labels. In order to detect molecules without electrochemical reaction, electrochemical impedance spectroscopy (EIS. In recent years, EIS has been able to make highly sensitive measurements as a sensing system especially in the development of biosensor technologies based on immunological based biomolecule interactions[6]. In EIS, the liquid interface properties and the binding parameters to the electrode surface can be measured. Since the EIS can measure the thickness or charge distribution (capacitance) of the electrode surface, it can be very sensitive to detect even very small changes that alter the electrical charge distribution on the electrode. Electrode surface resistance with EIS can be measured using potentiostat without any biochemical reactions. The color and turbidity properties of the measured fluid do not affect the measurement.
In the light of the above background, we immobilized HIF-1 antibodies which are capable of recognizing HIF-1 on the screen printed electrode surface (the sensor system). Thus, the new electrodes we developed had a more specific and rapid measurement capacity since a direct affinity based bioreceptor was used for the

target molecule HIF-1. The measurement of the binding of HIF-1 to the anti-HIF-1 on the electrode was performed by using surface-characterization EIS and the results were obtained with concentration-time-impedance function. In order to evaluate the validity of our measurement, results, procedure and performance characteristics were compared with the conventional ELISA technique.

Materials and Methods

Materials

All chemical materials were obtained from Sigma-Aldrich (USA). Electrochemical measurements were performed using the PalmSens3 potentiostat (Palmsens BV, The Netherlands). Screen printed gold electrodes (250 AT) were obtained from Dropsens (Spain). All chemicals were prepared using triple deionized water. Blood samples were taken from the control group and severe diabetic patients in whom amputation was planned. Serum were stored at -20°C until use. The study was approved by the Ethics Research Committee (16-9 /15) of
Ege University School of Medicine and supported by the Ege University Scientific Research Projects Commission (18-TIP-036).

Methods

This study included the archive blood of 30 patients between the ages of 18 and 100 who participated in the Diabetic Foot Council which is being held every Wednesday at Ege University Faculty of Medicine Orthopedic Policlinic within the scope of approval of the ethics committee numbered 16-9/15. HIF-1α levels were measured by both ELISA and biosensor we developed in the Department of Medical Biochemistry. The basis of this study is based on the immobilization of antibodies, specific molecules, to the surface after modification of the transducer surface and subsequent electrochemical examination of the surface due to the affinity between the HIF-1 molecule and the antibody. The bonding result of this affinity changed the electrical properties of the surface and after the change of electrical properties acceptable signals were obtained and electrochemical measurement was performed by electrochemical impedance spectroscopy and chronoimpedance. The electron transfer resistance was measured in redox probe solution, this measurement was calculated Randless circuit model [7]. R_2; represents the electron transfer resistance of the electrode surface. Since this resistance will change by binding of HIF-1α to the immobilized anti-HIF-1α on the electrode surface, the EIS values based on this have also changed. First of all, the gold nanoparticle modified electrode was placed in a cap containing water and ethanol (1:1) and left in a sonic bath for 2 minutes to remove impurities. Then, it was washed with pure water and dried under nitrogen gas. The gold nanoparticles were deposited on the surface by applying -200mV to the electrode immersed in a solution containing 200 ppm AuNPs. The electrode was then gently washed with distilled water and dried with nitrogen gas. AuNPs/BSA modification was carried out by the following modifications 50 µL of 1mg/mL BSA solution was placed on the surface of the gold nanoparticle coated electrode and incubated for 1 hour. AuNPs/BSA/PAMAM modification was carried out as follows; BSA on AuNPs was activated with 5% glutaraldehyde for 30 minutes and 5 mM 50 µL of PAMAM was dropped onto the surface and incubated for 1 hour. The electrode was washed with distilled water and gently dried with nitrogen gas. AuNPs/BSA/PAMAM/anti-HIF-1α modification was carried out via NH_2 ends on the AuNPs/BSA/PAMAM electrode by activating 5 % gluaraldehyde and subsequent incubated by dropping anti-HIF-1α on electrode. After the electrode was washed, the unbonded anti-HIF-1α removed. All modification steps were investigated by electrochemical impedance spectroscopy.

Results

All EIS curves of the electrode modification are shown in Figure 1 (Figure 1). The GNP modified electrode is shown in green. The BSA coating was used for covalent PAMAM modification by immobilization and pH stabilization of the surface of the electrode. As can be seen, an increase in electron transfer resistance was observed with coating of the surface. This is an expected result. The red curve shows the BSA layer. BSA has been used because of the ability of gold nanoparticles to attract proteins to the surface. Glutarladehide was used for covalent bonding of the next immobilization material (PAMAM) by activating amine groups on BSA. PAMAM-G5 is the fifth generation demdrimer class for electrode surface. This molecule is formed by branching ethylenediamine 5 times over the ethylene diamine nucleus and having amino groups at the end. This is also suitable for covalent bonding of BSA-PAMAM via glutaraldehyde. Since gold nanoparticles are nanomaterials, they are used in our biosensor system to facilitate electron transfer and increase surface area and provide active immobilization. PAMAM material was used to increase electrode surface area to immobilize more anti-HIF antibodies to increase sensitivity. In PAMAM bonding, because the surface is positively charged, it draws the negatively charged redox probe electrostatically to the surface, thereby increasing mass transfer, that is, Warburg impedance, increasing the electron transfer resistance on the electrode surface. The amino termini of PAMAM are reactivated with glutaraldehyde, making it suitable for anti-HIF binding. After activation of the PAMAM tips, anti-HIF was dropped onto the surface and covalently bonded to the surface. Since the anti-HIF immobilization increases the electron transfer resistance of the surface by forming an isolation layer, an increase in the impedance signal has occurred (Figure 1).

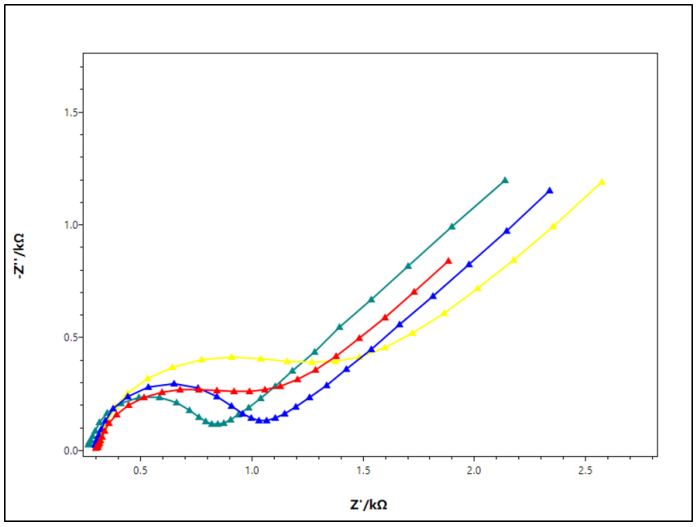

Figure 1. Red curve GNPE, Yellow curve GNPE / BSA, green curve GNPE / BSA / PAMAM, Blue curve GNPE / BSA / PAMAM / Anti-HIF1alpha modified electrode EIS curves.

Figure 2 shows that the conductivity of the gold nanoparticle electrode is high and the Warburg impedance is dominant. An increase in impedance occurred when the surface was coated with BSA. Although PAMAM coating is expected to increase in flowing impedance, since the PAMAM layer is positively charged at pH=7, the electron transfer resistance is reduced by pulling the negatively charged redox probe and increasing the number of redox probes that are electrochemically transformed per unit time. Anti-HIF1 binding also increases the electron transfer resistance. These procedures show that immobilization procedures are performed very successfully.

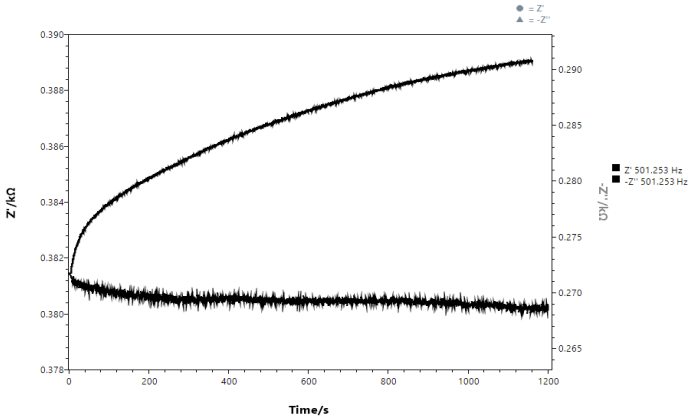

Figure 2. GNPE / BSA / PAMAM / AntiHIF1 a: 900mV 200Hz buffer and b: 900mV 200Hz HIF1.

After 40 seconds, the impedance curve began to gain linearity and continued linearly, although the binding speed was low. Therefore, the detection time is optimized for 10 minutes.

For calibration curves, 13 calibration curves were prepared to evaluate electrode performance characteristics such as GNPE/BSA/PAMAM/Anti-HIF1 modified biosensor Repeatability, selectivity, repeatability, linear spacing, LOQ, and LOD were performed (Figure 3A and B).

Comparison of the newly developed biosensor with the conventional ELISA method is given in Table 1.

Table 1. Comparison of the ELISA and the Biosensor

Parameters	HIF 1 Biosensor	ELISA
Sample	Serum	Serum
Linear Range	50 – 1000 pg/mL R^2= 0,9964	81,92 – 20000 pg/mL
LOD	14,45pg/mL (3,3(S(Ret=50pg/mL)/m)	<30pg/mL
LOQ	43,8 pg/mL (3,3(S(Ret=50pg/mL)/m)	-
Reproducible	R^2 = 0.9964 ± 0.0024 (n=13)	-
Repeatibility	1. (Serum n=3, 53,3): 51,6 ± 1,41 (pg/mL) 2. (Serum n=3,101,13) 100,3 ± 3,84 (pg/mL)	-
Detection Time	10 Minutes	5 Hours

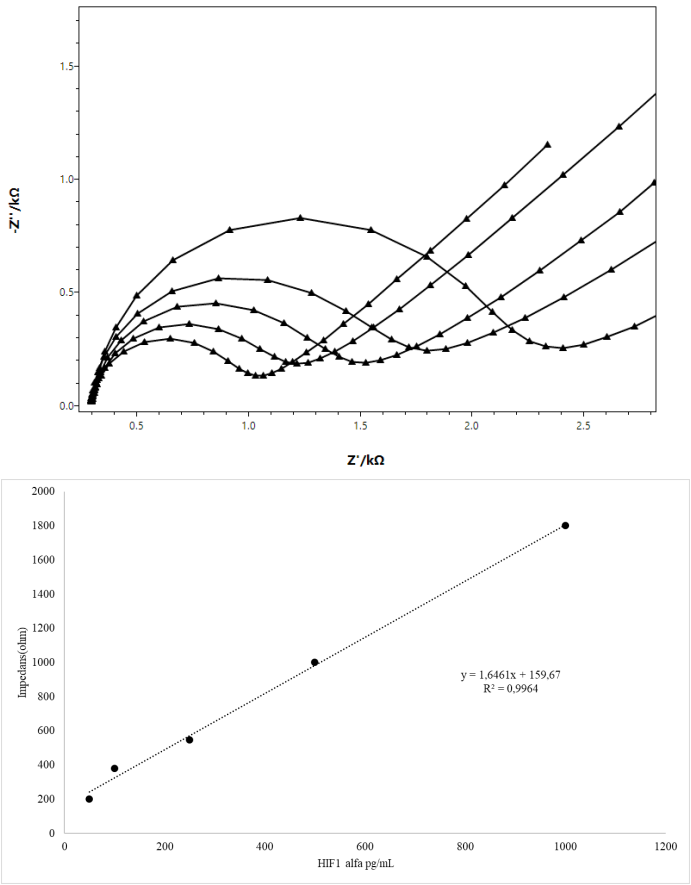

Figure 3. A: Impedance curve of the calibration curve of the HIF1 biosensor from 50 pg / mL to 1000 pg / mL. B: Calibration curve of the HIF1 biosensor (y-ΔRet, x-HIF (pg / mL.min)), R2 = 0.9964.

Supplementary Table 1 shows amputation and diabetes mellitus (DM) and amputation levels (Suppl. Table 1). HIF1 values (pg/mL) were also measured. HIF measurements using archive blood repeats from these patients in previous studies were performed using ELISA kit.

Conclusion

In this study, HIF1 alpha biosensor has been successfully developed for the first time in the literature. Our studies also include chrono-impedance studies to characterize the impedimetric detection time. The success of the biosensor in real serum samples also makes it possible to commercialize the biosensor after its development. The LOQ limit confirms that the calibration curve is close to the theoretical level. Selectivity studies using real serum samples and electrodes show good selectivity against serum samples. In the reproducibility study, when 13 calibration curves were prepared and the standard deviation of R2 values were taken, extremely low standard deviation showed that the system was both reproducible and reproducible. As a result, we have successfully developed our biosensor system by achieving our goal.

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Supplementary Table 1. HIF-1a levels in DM and amputation samples

Patient	DM Type	DM Duration (Year)	Amputation Level	HIF-1α (pg/mL)
Patient 1	Type-2	35	Finger	341.67
Patient 2	Type-2	6	Transmetatarsal	351.23
Patient 3	Type-2	1	Above Knee	673.77
Patient 4	Type-2	3	Transmetatarsal	64.23
Patient 5	Type-2	30	Finger	79.27
Patient 6	Type-2	32	Under the Knee	101.13
Patient 7	Type-2	25	Finger	54.67
Patient 8	Type-2	18	Finger	58.77
Patient 9	Type-2	45	Finger	43.73
Patient 10	Type-2	14	Under the Knee	60.13
Patient 11	Type-2	15	Chopart	57.4
Patient 12	Type-2	7	Under the Knee	53.3
Patient 13	Type-2	2	Finger	60.13
Patient 14	Type-2	22	Finger	102.5
Patient 15	Type-1	40	Under the Knee	639.6
Patient 16	Type-2	23	Under the Knee	49.2
Patient 17	Type-2	25	Transmetatarsal	82
Patient 18	Type-2	30	Finger	57.4
Patient 19	Type-2	20	Under the Knee	57.4
Patient 20	Type-2	40	Transmetatarsal	60.13
Patient 21	Type-2	27	Under the Knee	169.47
Patient 22	Type-2	2	Under the Knee	58.77
Patient 23	Type-2	16	Under the Knee	155.8
Patient 24	Type-2	20	Under the Knee	57.4
Patient 25	Type-2	4	Under the Knee	50.57
Patient 26	Type-2	15	Transmetatarsal	61.5

Reelin enzyme levels in patients admitted to emergency service due to suicide or self-harm attempt

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Abstract

Background: Every year more than one million people commit suicide worldwide. Suicide cases constitute 1-2% of the total global mortality. Reelin is an extracellular matrix glycoprotein and involved in the development of brain layers during embryogenesis. Reelin is linked with several psychiatric diseases but not investigated in the suicide cases.

Objectives: We aimed to investigate reelin enzyme levels among suicide patients in comparison to the heathy controls.

Materials and Methods: A total 86 suicide cases and 100 healthy controls were included in the study. Serum reelin levels were determined by using commercial Human ELISA kit. Demographic variables and clinic data were collected and analyzed.

Results: Body mass index and mean age among groups were significantly different. While the median reelin enzyme level of Suicide Group was 3038,31 (IQR:212,46-8044,21) ng/L and that of Control Group was 2271,20 (IQR:77,67-7647,83) ng/L. The difference was statistically significant (p <0.01).

Conclusions: According to the results of this study, reelin enzyme level of suicide patients was found to be significantly higher than normal cases. The importance and impact of our findings on suicide needs to be investigated across different populations.

Keywords: Suicide, Reelin, Emergency Service, ELISA, self-harm

Kendine zarar verme veya özkıyım girişimi nedeniyle acil servise başvuran hastalarda reelin enzim düzeylerinin araştırılması

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Özet

Giriş: Her yıl dünya çapında bir milyondan fazla insan intihar etmektedir ve intihar vakaları toplam küresel ölümlerin %1-2'sini oluşturmaktadır. Reelin hücre dışı bir matriks glikoproteinidir ve embriyojenez sırasında beyin tabakalarının gelişimine katılmaktadır. Reelin birçok psikiyatrik hastalıkla ilişkilendirilmesine rağmen intihar vakalarıyla ilişkilendirilmemiştir.

Amaç: Bu çalışmada intihar girişiminde bulunan hastaların reelin enzim düzeylerinin sağlıklı kontrollerle kıyaslanması amaçlanmıştır.

Materyal ve Metot: Çalışmada toplam 86 intihar girişiminde bulunmuş hasta ile 100 sağlıklı kontrol kullanıldı. Serum örneklerinde reelin düzeyleri, Human ELISA kiti kullanılarak kit prosedürüne uygun olarak incelendi. Demografik değişkenler ve klinik veriler de toplandı ve karşılaştırıldı.

Bulgular: Gruplar arasındaki vücut kitle indeksi ve yaş ortalaması farklılıkları istatistiksel olarak anlamlı bulunmuştur. İntihar Grubu'nun ortanca reelin enzimi değeri 3038,31 (IQR: 212,46-8044,21) ng/L iken, Kontrol Grubunda bu değer 2271,20 (IQR: 77,67-7647,83) ng/L olarak bulunmuş olup aradaki fark istatistiksel olarak anlamlıdır (p <0.01).

Sonuç: Bu çalışmanın sonuçlarına göre intihar hastalarında reelin enzim düzeyi normal vakalardan anlamlı derecede yüksek bulunmuştur. Bulgularımızın intihar üzerindeki önemi ve etkisi farklı popülasyonlarda da (daha yüksek veri ile) araştırılmalıdır.

Anahtar Sözcükler: İntihar, Reelin düzeyi, Acil Servis, ELISA, Kendine zarar verme

Introduction

Suicide is the self-injury with an aim to achieve death and is frequently encountered in emergency services and intensive care units [1]. Every year, more than one million people commit suicide worldwide and suicide cases constitute 1-2% of the total global mortality [2]. Most people who are on the verge of suicide tend to express their thoughts and intent. They use expressions that indicate desire to die and give signs that they feel worthless [3,4]. These are requests for assistance in suicidal cases. The thoughts and movements of people are limited just before the suicide as they consider suicide continuously and cannot perceive the other solutions for their problem(s) [3]. Suicide risk is increased 3-12 times among those with a history of psychiatric diseases compared to those without one [5]. Approximately 95% of people who have accomplished suicide have a history of psychiatric disease and the increase in frequency of suicide in some psychiatric diagnostic groups is noteworthy [4].

The gene encoding reelin is located on human chromosome 7 (7q22) and produces a protein with a molecular weight of 388 kDa. Reelin is an extracellular matrix glycoprotein involved in the development of brain layers during embryogenesis [6]. Reelin proteins are secreted from different regions of the brain, particularly Cajal-Retzius cells in the cortex and marginal zone of hippocampus. Reelin is not only secreted from the brain in the developmental process but is also secreted in adults from the glutamatergic granulated cells in the cortex and hippocampus GABAergic neurons in the cerebellum [7]. A number of studies indicated deficiency of the reelin in various neuropsychiatric diseases such as epilepsy, autism, major depression, lysencephaly, Alzheimer's disease [8–10]. However, to the best of our knowledge, there is no study targeting the relationship between reelin and suicide.

Therefore, in the present study we aimed to test the relationship between suicide and reelin protein.

Materials and Methods

This study was conducted after the approval of Fırat University Faculty of Medicine Clinical Research Ethics Committee with the decision number 06-06 dated 24/03/2015. A total of 86 patients who were suicide attempters and admitted to the Emergency Department of Firat University Medical School Hospital between March 2015 and November 2015 were included in the study and denoted as Suicide Group. In addition, a control group consisting of 100 healthy volunteers was formed and denoted as Control Group.

We initially recorded demographic data from all the individuals in healthy controls (Control Group) and suicide attempters (Suicide Group). We also measured and recorded body mass index (BMI), pulse, and respiration rate of all the cases.

We subsequently assayed blood serum reelin levels of all 186 individuals included in the present study. Reelin levels were assayed in serum samples according to the kit procedures using the Human ELISA kit (Rel Assay. Diagnostics, REF no1; E20160202016, LOT no1; 20160202, REF no2; E20160202017, LOT no2; 20160202). Samples were not diluted. Absorbances were read spectrophotometrically at 450 nm on ELX800 ELISA reader. In plate washing, Bio-tek ELX50 (BioTek Instruments, USA) was used as an automatic washer. Results were expressed as ng/L. The measuring range was 37 ng/L-7000 ng/L and the minimum measurable level was 15.23 ng/L.

After the data were collected, statistical analyses were performed using the statistical analyses program SPSS 21.0. Kolmogorov-Smirnov and Shapiro-Wilk normality tests were employed to test the deviations from the normality. Numerical data were expressed as mean ± standard deviation and qualitative data were expressed as percentages. In addition, data that did not fit into the normal distribution were presented as Median (Interquartile range, IQR). Student t-test was used to test differences between means of two groups with the continuous variables that were normally distributed. Chi-Square test was used to test non-random distribution of categorical data among groups. Man-Whitney-U test was used to compare non-parametric paired groups. A p-value of <0.05 was considered significant throughout the analyses.

Results

Of the 186 patients included in our study, 86 were suicide attempters and 100 were healthy controls. Among 86 suicide attempters, 52 (%60.5) were females and 34 (%39.5) were males. The control group consisted of 54 females and 46 males. The mean age of Suicide Group was 28.14 (±10.04) years and that of Control group was 32.52 (±13.71) years. We conducted a Chi-square test was used to assess the gender distribution among groups and the results revealed that the difference was not significant (p=0.69) (Table 1).

Although there was a statistically significant difference between the groups in the mean BMI (p = 0.001), age (p = 0.001), and respiratory rate (p <0.001), these values were within the limits accepted as clinically normal.

Table 1: Comparison of demographic and clinic data of Suicide Group and Control Group.

Parameters	Suicide Group	Control Group	P
N (Female/Male)	86 (52/34)	100 (54/46)	0.690
Age (years)	28.14±10.04	32.52±13.71	0.001
BMI (kg/m^2)	23.28±4.47	24.93±3.38	0.001
Pulse (beats per min)	90.65±21.76	84.92±14.78	0.112
Respiration Rate (breaths per min)	17.80±3.17	14.96±1.20	<0.001

In our study, the median reelin enzyme level was found to be 3038.31 ng/L (IQR: 212.46-8044.21 ng/L) in Suicide Group and as 2271.20 ng/L (IQR: 77.67-7647.83 ng/L) in Control Group. The difference in the reelin enzyme levels between the two groups was found to be significantly different (p <0.0001) (Table 2).

Table 2. Comparison of reelin levels in patients attempting suicide and healthy control group.

Parameters	Suicide Group	Control Group	P
N (Female/Male)	86 (52/34)	100 (54/46)	0.690
Reelin Enzyme Median (IQR ng/L)	3038.31 (212.46-8044.21)	2271.20 (77.67-7647.83)	0.001

Discussion

Suicide is an increasing public health problem in our country as well as all over the world [11]. A number of earlier studies targeted the demographic data from the suicide cases in Turkey to deduce the general pattern. Önsüz et al. [12] evaluated 1566 patients with suicide attempts in the emergency department and reported that 78.9% were women and 1109 of all patients were in the 15-34 age group. In a 10-year retrospective study, 2988 out of 4569 patients admitted to the emergency department for suicide attempt were female (65.4%). The study also concluded that the mean age of females was 24.5±10.1 years and the mean age of males was 29.5±13.2 years [13]. Kara et al. [14] found that 74% of 1036 patients were females in their study on the evaluation of patients admitted to the emergency department due to intoxication. They also reported that 60% of all patients were in the 15-24 age group [14]. Ersoy et al. [15] found in their retrospective study that the female ratio was higher (72.8%) and the mean age of all patients was 27±13.8 years. The demographic data reported here showed similar pattern to the aforementioned literature in terms of gender and age.

An intensive amount of research has been devoted to understanding the biochemical pattern in suicidal patients. However, the role of reelin in the suicide cases has not yet been investigated. Here, we evaluated demographic data from the patients who attempted suicide in respect to reelin levels and found a negative correlation with age and body mass index. In a study on patients with autism, Camacho et al. [16] found that the reelin protein is expressed and stored in Cajal-Retzius cells developing in the cereal cortex and cerebellum. While these cells are reported to be present in the developing cerebral cortex, most of these cells have been detected to have apoptosis and a very small group of them are alive in the progressed age groups. Therefore, as in our study, the level of reelin was found to be higher in individuals at younger ages [16].

The role of reelin in the innate psychiatric diseases have been investigated in depth. One of the most important mechanism of action of the reelin is synaptogenesis, structural and biochemical neuroplasticity in dendritic endings of axons in the hippocampus in the human brain [17]. Reelin level deficiency is attributed to disruption of neuroplasticity and regulation of gene expression from the endoplasmic reticulum, protein production and their posttranslational modification in peripheral tissues [17]. Similarly, in our study, an inverse relationship was found between the reelin level and body mass index, and it was found that the reelin level decreased as the body mass index increased.

More than 100 markers have been identified in the studies conducted to detect a possible biological marker in the brains of schizophrenia patients and reelin and glutamic acid decarboxylase (GAD) have been frequently studied in this context [18–20]. Both proteins are expressed in cortical GABAergic neurons. While GAD plays a role in GABA synthesis, the extracellular matrix protein, reelin, binds to dendrites and plays a role in long term potentialization, which is important in learning and memory. Low levels of both reelin and GAD1 mRNAs are among the most frequent findings in schizophrenia brains [8,19,21–24]. This suggests that the regulation of the GABAergic system is impaired in schizophrenia. This disorder has been reported to be associated with impaired working memory in patients with schizophrenia [25].

In the study of 10 male and 8 female patients diagnosed with the schizophrenia, Hornig et al. [26] investigated reelin enzyme level while comparing them to a total of 18 (9 females/ 9 males) healthy controls. ELISA and Western Blot were used in the study and both methods were compared. In both methods, it was found that reelin enzyme level increased significantly in schizophrenia patients. They claimed that the drugs patients had taken increased the level of the reelin [26]. Similarly, Fatemi et al. found that the concentration of blood reelin in patients with schizophrenia increased compared with the healthy control group [8].

Reelin levels were evaluated in a wide range of neurological and neuropsychiatric diseases such as depression, schizophrenia, Alzheimer's and different results were obtained in each disease. The levels of reelin in postmortem brain tissues were investigated in schizophrenia patients and this enzyme was found to have decreased in patients with schizophrenia. In our study, the blood serum reelin levels were found to have elevated similar to Hornig et al. [26] and Fatemi et al. [8]. However, in both studies, it is stated that there is not enough data to explain the molecular mechanism of the increase.

There is a general lack of empirical information between suicide and reelin. We believe that more conclusive data could be conceived with a wider patient population. In addition, it was concluded that this study will be reference point for the future studies.

Conclusion

According to the results of this study, reelin enzyme level of suicide patients was found to be significant factor for suicide. The importance and impact of our findings on suicide needs to be investigated and targeting role of reelin in experimental animal studies will be an interesting research venue.

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