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"12th Annual Conference of Pakistan Society of Chemical Pathology, ChemCon 2022"

Metabolomics: Revolutionizing Chemical Pathology

ABSTRACT BOOK

IFCC International Federation of Clinical Chemistry and Laboratory Medicine

PAKISTAN SOCIETY OF CHEMICAL PATHOLOGISTS PSCP

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"12th Annual Conference of Pakistan Society of Chemical Pathology, ChemCon 2022"

Metabolomics: Revolutionizing Chemical Pathology

Organized by

Pakistan Society of Chemical Pathology (PSCP), Karachi Chapter

under the auspices of

International Federation of Clinical Chemistry & Laboratory Medicine (IFCC)

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12th Annual Conference of Pakistan Society of Chemical Pathology, ChemCon 2022

Metabolomics: Revolutionizing Chemical Pathology

Organized by Pakistan Society of Chemical Pathology (PSCP), Karachi Chapter Under the Auspices of International Federation of Clinical Chemistry & Laboratory Medicine (IFCC)

Date: 25th, 26th November 2022
Venue: Pearl Continental Hotel
Club Rd, Opposite PIDC، Civil Lines, Karachi, Sindh

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FOREWORD

It gives us an immense pleasure to share this special Journal of Pakistan Medical Association (JPMA) supplement, published in line with the 12th Annual Conference of Pakistan Society of Chemical Pathology, ChemCon-2022. The theme of this conference is 'Metabolomics: Revolutionizing Chemical Pathology'. A pre-conference workshop will be conducted to introduce the recent technological advancements in state-of-the art metabolic and profiling techniques used in metabolomics. International experts will share their perspective on the diverse applications of metabolomics in laboratory medicine and national experts have addressed the subject matters related to Metabolomics applications in research, education, and clinical service in Pakistan's context.

Selection of best abstracts was challenging for the scientific committee as we received an enormous response from the Chemical Pathology community of Pakistan. A total of forty-four abstracts were accepted. After a rigorous evaluation process, twelve were selected for oral and the rest were accepted for poster presentations. From across the country chemical pathologists have shared their research findings on diverse topics which are presented in this abstract book.

The scientific content of this conference particularly encourages the interaction of residents, technologists, and pathologists with the established national and international academic community to present and to discuss new and current work.

We look forward to welcoming you all and we hope you will take an active participation in the 12th Annual ChemCon-2022, from November 25-26th, 2022.

Hafsa Majid

Chair Scientific Committee
ChemCon-2022 'Metabolomics:
Revolutionizing Chemical Pathology'

EDITORIAL

Metabolomics: Revolutionizing Chemical Pathology in Pakistan through ChemCon 2022

Lena Jafri
Chair Organizing Committee, ChemCon-2022 'Metabolomics: Revolutionizing Chemical Pathology'

Metabolomics is one of the most robust bioanalytical approaches that allow obtaining a picture of the metabolites during a biological process in humans and is thought as a phenotyping tool.[1] With the advent of precision medicine, clinical metabolomics is on the spotlight for being able to provide clinicians with novel sets of predictive and prognostic biomarkers for diseases in addition to quantifying treatment response to medications at an individualized level.[2]

Developments in the analytical techniques in chemical pathology and metabolomics are the source of the rapid evolution of a new omics era.[3] Although the science of metabolomics in Pakistan is in infancy but on situation analysis of the clinical laboratories in Pakistan there have been some small advancements.[4-8] With the advent of Gas Chromatography Mass Spectrometry, Liquid Chromatography Mass Spectrometry (LC-MS), and Fourier Transformed Infrared Spectroscopy for clinical application in clinical laboratories of Pakistan diagnosis and management of many rare inherited metabolic disorders (IMD) is now possible.[9,10] The 'Dried Blood Spot Metabolic Profile' has been introduced on LC-MS/MS for newborns' screening. With one blood spot multiple amino acids, acylcarnitines and succinyl acetone can be quantified and more than 30 IMDs can be identified.

To facilitate understanding of metabolomics and to enhance the science of metabolomics amongst chemical pathologists a monthly activity of 'Metabolomics Book Club' has been recently initiated. Novel information of metabolomics is disseminated to all attendees, and this is followed by discussions.[11]

A working group of the Pakistan Society of Chemical Pathology, Pak-IMD-Net, was formulated to strengthen the clinical laboratories on application of metabolomics.[12] The year 2021 was celebrated as the year of metabolomics by Pak-IMD-Net. The educational meetings in 2021 revolved around two omics domains: analytical techniques, (mass spectrometry) and multivariate data analysis software (Collaborative Laboratory Integrated Reports -in collaboration with Mayo Clinic, USA). Since its inception in 2019, through educational activities and trainings, fulfilling its goal of capacity building, Pak-IMD-Net has improved the diagnostics and patient care in our country.[13]

To raise awareness of rare metabolic disorders, under the auspices of International Federation of Clinical Chemistry & Laboratory Medicine (IFCC), the chemical pathology fraternity came together in 2020 for the first time. A multidisciplinary conference was conducted to discuss local challenges and solutions of metabolomics for rare diseases. International experts from USA, Italy and New Zealand addressed the symposia and facilitated workshops to train the local chemical pathology work force for establishment of Newborn Screening in Pakistan.

Since past decade, the chemical pathologists of Pakistan have stood for open science especially for 'metabolomics'. Since 2020 several new and important milestones in the history of metabolomics in Pakistan. Our aim is to discuss all these advancements and challenges in the local context of Pakistan in this forthcoming conference- ChemCon 2022. Horizon scanning and scenario planning in relevance to metabolomics in chemical pathology will be carried out in this exciting scientific meeting. This thematic conference will help motivate a generation of analytical scientists in Pakistan, which will help move the field forward with greater momentum.

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PLENARY SESSION ABSTRACTS

Abstract-01

International Federation of Clinical Chemistry & Laboratory Medicine (IFCC): 70 Years of Global Leadership with New Vision/Strategic Direction Post-pandemic

Khosrow Adeli

President IFCC, Professor Pediatric Laboratory Medicine,
The Hospital for Sick Children, University of Toronto, Ontario, Canada

IFCC is the leading worldwide organization in the field of laboratory medicine and continues to focus on its mission of "advancing excellence in laboratory medicine for better healthcare worldwide". Over the two years, it has been making significant strides by promoting the value of laboratory medicine, impacting healthcare delivery and patient outcomes, contributing to global lab quality, becoming the largest provider of free distance learning in the field of laboratory medicine, and aiding in the fight against the COVID-19 pandemic. Several projects have been initiated to meet the goals of the strategic plan. First, IFCC is working to gather evidence to demonstrate the value of laboratory medicine in healthcare delivery, particularly in the context of clinical decision-making. Projects have also been initiated to directly impact healthcare delivery, such as the global newborn screening programme, which aims to support newborn screening in developing countries.

To improve global lab quality, the IFCC is also planning to initiate programmes to assist clinical laboratories in improving internal and external quality assurance (IQC and EQA) in developing countries as well as create a global reference interval database to support reference interval harmonisation. Another key component of the strategic plan is to improve distance learning opportunities for IFCC members around the world, and thus the IFCC has been hosting live biweekly or monthly webinars on a variety of laboratory medicine topics. Last but not the least, IFCC has been very active in supporting the fight against the COVID-19 pandemic by summarising, critically reviewing, and disseminating the most up-to-date, evidence-based information about the novel coronavirus. Several evidence-based guidelines have also been published by IFCC in the areas of lab biosafety and laboratory testing to support the important work of clinical laboratories around the world. Ultimately, in all our endeavors, IFCC is committed to encouraging and supporting a culture of innovation and increasing productivity. In this brief presentation, I will provide information about IFCC projects, their potential impact, progress made so far, and future directions.

Keywords: COVID-19, quality assurance, IFCC.

Abstract-02

Metabolomic, Immunological, and Biochemical Profiling of Adolescents with Obesity and Insulin Resistance

Siobhan Wilson, Victoria Higgins, Khosrow Adeli

Molecular Medicine, Research Institute, The Hospital for Sick Children, and Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, ON, Canada

Background: The increased risk for cardiovascular disease (CVD) and Type 2 Diabetes (T2D) seen in adolescent obesity is due in part to lipid and lipoprotein abnormalities. Indeed, postprandial dyslipidaemia is an independent risk factor for CVD. Postprandial, opposed to fasting, dyslipidaemia is also evidenced to better reflect metabolic activity, as the fed state predominates throughout the day. In addition, key intestinal regulators of lipid metabolism are reportedly maladaptive and associated with postprandial dyslipidaemia in adolescent obesity and insulin resistance (IR). Further, obesity contributes to IR and CVD pathogenesis, in part, by inducing systemic inflammation. The objectives of the current study were to characterise the fasting and postprandial profiles of inflammatory and metabolic markers in adolescents with obesity and IR, and to assess their role in the pathogenesis of postprandial dyslipidaemia.

Methods: Adolescents with normal weight (NW; N=15), with obesity and mild IR (N=20), and with obesity and severe IR (N=10) were recruited. All participants (12-<19 years) underwent a 6-hour oral fat tolerance test (OFTT) in the SickKids hospital investigational research unit. Inflammatory and metabolic markers were assessed via various immunoassays and nuclear magnetic resonance (NMR) spectroscopy, respectively. The three study cohorts were compared for all analytes using a two-way ANOVA.

Results: Among inflammatory profiling, levels of interleukin (IL)-6 were significantly elevated in both obese groups at fasting (P<0.001), which was maintained postprandially compared to the NW response (main effect of group: P<0.05). The between-group difference in postprandial responses observed in other studied cytokines did not reach, although few approached (i.e., tumour necrosis factor-?, IL-18), statistical significance. Levels of serum calprotectin demonstrated a significant interaction between study group and OFTT time-points (P<0.005). Correlational analyses revealed strong (-0.5> Spearman rho > 0.5) and significant (P<0.05) associations between tumour necrosis factor-?, IL-6, and IL-8 and an atherogenic lipid/lipoprotein phenotype, adiposity, and IR, while IL-1B, IL-18, and monokine induced by interferon-y were strongly associated with bile acid species. In metabolomic profiling using NMR, valine, leucine, and isoleucine (branched-chain amino acids, BCAAs) and alanine were significantly elevated in obesity, particularly those with severe IR (P<0.05). Importantly, despite elevations among both obese groups, the postprandial response to a high-fat meal was blunted therein (group*time interaction P<0.005). Furthermore, BCAAs and alanine were positively (rho>0.7, P<0.05) associated with IR.

Conclusions: Adolescents with obesity and IR exhibit significant fasting and postprandial dysregulation of several inflammatory and metabolic markers integral to lipid metabolism. Inflammatory proteins, IL-6 and calprotectin, were elevated in obesity and IR, suggesting a state of chronic inflammation in such subjects that may interfere with postprandial lipid metabolism. Amino acid levels demonstrated a stepwise increase across study groups and a blunted postprandial response, suggesting a potential mechanism of involvement in both obesity- and IR-related pathogenesis that may involve a complex interplay with postprandial lipid handling. These data may offer novel subclinical biomarkers for early metabolic and cardiovascular diseases, such as postprandial dyslipidaemia, in at-risk adolescents. Future research should seek to determine the predictive capacity of the studied biomarkers for postprandial dyslipidaemia and future CVD and T2D.

Keywords: Metabolomics, Adolescents, Obesity, Insulin Resistance.

Abstract-03

Clinical Applications of Volatolomics

Sergio Bernardini
Chair Emerging Technologies Division Executive Committee Professor University of Tor Vergata, Rome, Italy

Metabolomics requires the identification of a large number of molecules in body fluids. For the scope, complex instruments able to sort the molecules according to their properties are necessary. Sensors introduce a separation principle based on the affinity with sensitive materials. This concept is fully developed in sensors arrays where sensors functionalised with different sensitive materials are used to characterize complex samples.

The selectivity of individual receptors is not absolute, but the ensemble of sensors provides a fingerprint that can be selective enough to identify the patterns of metabolites. This approach is analog to the combinatorial selectivity of natural olfaction. Arrays of gas sensors, called electronic noses, have been extensively applied to breath analysis, in particular for cancer diagnosis. Recently, olfaction inspired cross-reactive sensor arrays for rapid profiling of chemical and biological systems using synthetic receptors have been made. The implementation of the combinatorial selectivity principle requires the development of sensors with sufficiently different selectivity pattern. At the same time, sensors fabrication has to be reasonably cheap and fast in order to produce arrays with a sufficiently large number of integrated devices. For the scope, several approaches are available. Porphyrinoids are an example of sensitive materials whose broad-selectivity can be modulated changing the molecular components, such as the molecular framework, the metal ion and the peripheral compounds . Arrays of sensors have been used to assess food and air quality and to detect lung and kidney cancer, asthma, and infectious diseases, Covid-19 included.

Keywords: Porphyrinoids, sensors, volatolomics

Abstract-04

Metabolomics in Chemical Pathology: Where and When?

Elie Fux

Chairman of the IFCC metabolomics working group
Group Head Mass Spectrometry Applications, Roche Diagnostics / IFCC Emerging technologies, Germany

Comprehensive metabolic profiling, using metabolomics, has become widely accepted as a dynamic and sensitive measure of the phenotype at the molecular level, placing the technology at the forefront of biomarker and mechanistic discoveries related to pathophysiological processes. Primary metabolites involved in cellular function, maintenance, differentiation, growth, and death are of particular interest for biologists and clinicians.

This presentation will cover the concepts of metabolomics and the recent advances of analytical methods used to generate metabolomics data. Two methodological approaches will be presented:

1) Untargetted metabolomics, a data-driven and hypothesis-generating approach involving comprehensive profiling of as many metabolites as possible to enable disease-associated patterns to be deduced for potential clinical use, and

2) Targetted metabolomics, involving analysis of a selected set of chemically characterised and biochemically annotated metabolites from a single or multiple pathways that are associated with a particular pathology or clinical condition of interest.

Examples of applications of metabolomics in the field of clinical chemistry will be discussed as well as the potential of using the technology to develop new diagnostics and prognostics procedures.

Keywords: untargeted metabolomics, targeted metabolomics, technologies.

Abstract-05

Situation Analysis of Metabolomics in Research in Chemical Pathology — a Pakistani Perspective

Sibtain Ahmed,[1] Adnan Mustafa Zubairi[2]

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Metabolomics is the study of unique chemical fingerprints that specific cellular processes leave behind. Generally, metabolomics includes study of small molecules (<1.5 Kda) and is also called "Small Molecule Profiling". Metabolomics provides analysis of both "endogenous" e.g., amino acids, lipids, cofactors, nucleotides, sugars, hormones, etc. as well as "exogenous" metabolites e.g., drugs, toxins, environmental contaminants, pesticides, herbicides, etc. The commonly used samples are biofluids like saliva, blood, urine and faeces. Metabolic analyses at first relied upon nuclear magnetic resonance (NMR), although recent improvement in the field of mass spectrometry (MS) and Tandem MS has opened broader horizons for research, service and education. In this overview, the research domain of Mass spectrometry was explored with Chemical Pathologists perspective from Pakistan. As a baseline, search engine Pakmedinet was used including key words "Metabolomics", "Mass spectrometry", "Pakistan" with no date and time restrictions and the search was further refined and only articles having Chemical Pathologists as authors were included. Review articles, abstracts only, case reports, case series and frequency reports were excluded. The literature review yielded a total of 1167 articles of which 1155 were excluded and 12 were included. Majority of the publications were case series and retrospective lab data with no significant efforts focused on high yield exploratory research using MS. The review revealed MS utilization for method development and biomarkers evaluation from the basic biological sciences group and pharmaceutical industry in Pakistan, but scattered efforts in silos lacking clinical utilization in liaison with Chemical Pathologists. It is high time for Pakistan to utilize MS advances for research and development, as it is the driving force behind service and education. As a way forward, newborn screening, inherited metabolic disorders diagnostics, heavy metals analysis and toxicology are the domains to pursue research for Chemical Pathologists in the country. To serve the purpose, formulation of working groups, establishing liaisons with institutes having expertise and provision of funding opportunities under the umbrella of PSCP is required.

Keywords: Metabolomics, Mass spectrometry, Pakistan.

Abstract-06

Situation Analysis of Metabolomics in Clinical Service in Chemical Pathology — a Pakistani Perspective

Sheharbano Imran,[1] Aysha Habib Khan[2]
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[2]Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan

Accurate and timely diagnosis at an affordable cost is backbone of any health care system. For clinical laboratories, quantifying multiple metabolites with high sensitivity and accuracy by using a single method is a great advantage. Metabolomics has made significant growth in diagnostic and predictive medicine due to its role in diseases identification, severity, and prognosis in a single method. Considering a very close relationship to the phenotype than any other omics discipline, metabolomics is considered as prospects of chemical pathology in many fields including toxicology, pharmaceutical, Nanotechnology, predictive medicine, nutraceuticals, and regenerative medicines etc.

Recently, using advanced analytical methods and bioinformatics, targeted weakness in metabolomics have largely been overcome and inherent technical limitations have also been minimised. High hypothesis-generating potential and translational skill of metabolomics is now established, and panels of biomarkers have been defined by some clinical labs in US. However, pre-analytical, analytical and post-analytical limitations hamper its availability for translating into clinical use in Pakistan. This includes need for different platforms and experts (including analytical chemists, biologists, statistician, data scientists and bio-informaticians) to provide a comprehensive metabolome coverage reliably and ethically.

Pakistan is going through a demographic transition from communicable to non-communicable diseases and is currently facing several severe economic challenges including resource-sensitive operational challenges for business. In the current situation, translation of metabolomics into practice for precision medicine and precision prevention in Pakistan is a daunting task. Major efforts are needed to develop the analytical infrastructure, broad incorporation of machine learning techniques and systems to assist in disease diagnosis and treatment and predict prognosis in precision medicine. An effort is needed against discrimination in access to treatments.

We searched the literature to identify the utility of metabolomics in chemical pathology laboratories in Pakistan. A cross sectional survey was performed to assess the availability of metabolomics, computational methods, and resources in chemical pathology laboratories in Pakistan. We also proposed a way forward for translating research work into practice.

Keywords: toxicology, pharmaceutical, nanotechnology, predictive medicine.

Abstract-07

Situation Analysis of Metabolomics in Clinical Service in Chemical Pathology — a Pakistani Perspective

Hafsa Majid,[1] Aamir Ijaz[2]
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The field of metabolomics has matured over the past decade and is being increasingly used in clinical settings. With technological advances its clinical, especially diagnostic applications are increasing, but the number of competent experts in this field are not increasing at the same pace, the main reason being the insufficient attention towards the capacity building of the practicing and trainee Pathologist. We did a need assessment survey to understand, the where do we stand as far as the metabolomics education or training in Pakistan is concerned, the need for a curriculum, the existing state of education, biochemical genetics training, if specified, the length of the course and the competencies that should be covered.

This was prompted by the need to improve the competence of Pathologist on biochemical genetics and Inborn metabolic diseases, an area not fully addressed in the postgraduate curriculum. This survey aimed to guide the development of fit-for-purpose curriculum for the Chemical Pathology Postgraduate trainees.

Most of the survey respondents agreed that there is a need to strengthen the existing curriculum for the postgraduate trainees of Chemical Pathology. Many also responded to initiate formal academic programmes, conduct standalone courses or workshops to fill the current deficiencies in our training, create opportunities for formal internships or attachments and facilitate trainees by building local or international networks of training facilities.

It is high time to initiate discussions within the Chemical Pathology community of Pakistan to consider a formal academic programme on Metabolomics and strengthen the existing postgraduate programme to achieve high training standards as technologies evolve.

Keywords: Pakistan, training, metabolomics

Abstract-08

Reels on Metabolomics from Pakistan

Imran Siddiqui, Hafsa Majid, Lena Jafri
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With changing times, the modes of healthcare content delivery are also changing as people are moving towards visual data delivery, infographics and short duration videos. Literature supports that the visual interventions take less time in comprehending and are more effective tools in getting across your message.

Short videos or reels are one such tool that have great potential for raising awareness or conveying your point of view. In ChemCon 2022 we are introducing 'Reels on Metabolomics' to create noise and promote the science of metabolomics in chemical pathology fraternity and beyond. We invited Chemical Pathologists and personnel involved in Pathology Education to share short videos of few seconds related to different topics on metabolomics.

Pathologists shared their experiences and achievements, or other interesting information regarding the significant work on the Metabolomics. Those involved in diagnostic service delivery, shared their experience of establishing metabolomics services for screening, diagnosis and/or monitoring of inherited metabolic disorders, toxicology, therapeutic drug monitoring etc, and the way by which, it is facilitating the clinicians across Pakistan. Some of the reels are depicting scope of metabolomics application in personalised and precision medicine, opportunities for training on metabolomics, both locally and internationally, and the value metabolomic services have added to the healthcare landscape of Pakistan.

Now there is a need to develop actionable plans of bringing metabolomics in Chemical Pathology training and laboratory clinical services across. We hope and wish that these short videos can play an important role in demonstrating the current Pakistani perspective on metabolomics to our fraternity and beyond.

Keywords: Metabolomics, Pakistan, pathologist, reels, videos.

WORKSHOP ABSTRACTS

Abstract-09

Metabolomics in the Screening and Diagnosis of Inherited Metabolic Disorders

Fatima Kanani
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Metabolomics is a fast-mushrooming field in Chemical Pathology. It is being increasingly recognized as a potentially powerful tool for precision medicine. However, there are very few institutes in Pakistan which have the facility for it, albeit, limited. The major challenges in this regard are the high cost and expertise required. A need for capacity building and knowledge enhancement was foreseen as the first step to meet the challenge, and an execution plan via scientific conferences, webinars and workshops was envisaged.

Both targetted and un-targetted metabolomics have diverse applications in clinical diagnostics. There is a need to develop expertise on the principles and applications of the different techniques used in metabolomics such as nuclear magnetic resonance (NMR) and Mass spectrometry (MS) with a focus on their strengths and limitations. Volatolomics, or study of volatile metabolites, is a closely related field. The human volatolome forms a distinct pattern in physiological and pathological processes which can aid in diagnosis of certain metabolic disorders, as for example in breath or sweat analysis. Lipidomics, the study of cellular lipids, is a relatively more recent field which has gained increasing popularity because of its role in screening and monitoring of mitochondrial defects or fatty acid oxidation disorders by mass spectrometric analysis.

Thus, there is a growing role of metabolomics, volatolomics and lipidomics in screening and diagnosis of inherited metabolic disorders. It is pertinent for Chemical Pathologists fraternity to not only be acquainted with the basic principles and techniques used in them but also to keep abreast with the latest technological advances in these fields.

Keywords: metabolomics, lipidomic, inherited metabolic disorders.

Abstract-10

Analytical Techniques applied in Metabolomics

Zujaja Hina Haroon
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Metabolomics is the comprehensive study of small-molecule metabolites. Obtaining a wide coverage of the metabolome is challenging because of the broad range of physicochemical properties of the small molecules. To study the compounds of interest, two leading analytical platforms, nuclear magnetic resonance (NMR) and mass spectrometry (MS) coupled with separation techniques (LC, GC), are used. Both instruments are capable of reproducible and high throughput measurements of large numbers of metabolites. The choice for a given technique is influenced by the sample matrix, the concentration and properties of the metabolites, and the amount of sample. Each instrument has advantages and limitations, and no single instrument or instrument method can detect all metabolites present in a metabolome. Due to the metabolome's complexity and the diverse properties of metabolites, multiple instrument methods or multiple different instruments are required to provide the greatest number of metabolites detected. The combined use of modern instrumental analytical approaches has unraveled the ideal outcomes in metabolomics. It is beneficial to increase the coverage of detected metabolites that single-analysis techniques cannot achieve. Continued development of these analytical platforms will accelerate widespread use and integration of metabolomics into systems biology. Here, the application of each hyphenated technique its strengths and limitations will be discussed.

Keywords: nuclear magnetic resonance, mass spectrometry, metabolites

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Abstract-11

The Diagnostic Properties of the Human Volatolome

Corrado Di Natale

University of Rome Tor Vergata, Rome, Italy

The volatile fraction of the metabolome, the volatolome, is gaining a growing interest because of a supposed simplicity of samples collection, the intrinsic non-invasiveness of measurements and the wide availability of analytical methods. Studies evidenced that patterns of volatile organic compounds (VOCs) have been shown to be related to a vast range of phenomena observable in vitro, even at single cell level, and in vivo. Several instrumental techniques are available for the analysis of volatolome. Gas chromatograph and mass spectrometers provides a thorough investigation about the volatolome composition. On the other hand, portable and easy to use instruments based on sensors arrays (so-called electronic noses) are also becoming available. Electronic noses have been demonstrated to be sufficiently sensitive and selective to identify diseases analyzing various human samples such as breath, urine, and sweat.

Human volatolome is very complex. It contains about 2000 different compounds and all the major chemical families are represented; the largest diversity of compounds is found in breath. Independently from the nature of the analytical technique, the analysis of volatolome always returns a fingerprint, namely a multivariate data. In this the principle of volatolomics are reviewed, major instrumental techniques are introduced, and results obtained in selected study cases are discussed.

Keywords: Volatolome, multivariate data, mass spectrometers.

Abstract-12

Metabolomics and lipidomics in diagnosing inborn errors of metabolism

David Friedecky

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In recent years, omics techniques targetting low molecular weight analytes have found their place in the search for biomarkers of known diseases and in routine diagnostics. With the continuous refinement of metabolite and lipid analysis methods and their application in the study of various diseases, metabolic disruption has been shown to play a key role in many pathophysiological and patho-biochemical processes. Targetted and non-targetted approaches then provide a new comprehensive view of metabolic/lipid profiles corresponding to alterations in a wide range of diseases. Inborn errors of metabolism (IEM) comprise a large group of diseases caused by enzyme deficiencies that can be studied using metabolomics or lipidomics. Both have been applied to a number of IEM in recent years for both study and diagnostic purposes. This presentation will review the use of lipidomics in disorders of beta fatty acid oxidation, peroxisomal disorders, mitochondrial disorders, hyperuricaemia, and others. Although metabolomics has been the main domain of study and diagnosis of IEM, lipidomics has given us a new "outside-in" perspective on this issue in recent years. In the years to come, lipidomics may help us to better understand the patho-biochemistry of these diseases with better treatment monitoring options.

Keywords: Inborn errors of metabolism, metabolic, lipid profiles.

SCIENTIFIC SESSION ABSTRACTS (ORAL)

Abstract-13

Evaluation of heart fatty acid binding protein (H-FABP) as a diagnostic marker in acute coronary syndrome

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Introduction: Heart Type Fatty Acid Binding Protein (H-FABP) is biomarker suggested for early detection of Acute Coronary Syndrome (ACS). Early diagnosis and management are critical as it could prevent and reduce morbidity and mortality. The study aimed to determine the diagnostic accuracy of H-FABP as a biomarker of ACS by using high sensitivity Troponin I(hs-TnI) and/or ECG findings suggesting ischemia, as gold standard.

Methods: The study was conducted from May 2020 to November 2020 at Dr. Ziauddin University Hospital in Karachi. Blood samples of 154 patients were collected, who presented to emergency department or admitted to Cardiac Care Unit of more than 18 years of age with symptoms suggesting angina as pain in chest, left arm/lower jaw, increased heart rate and shortness of breath. Patient's data regarding age, gender, duration of chest pain onset, kidney disease, diabetes mellitus, hypertension and smoking was collected. Serum H-FABP was analyzed on Quantitative Fluorescence immunoassay Analyzer. Diagnostic accuracy statistics for H-FABP were calculated with the help of contingency tables taking hs-TnI and/or ECG findings as gold standard.

Results: Overall H-FABP showed sensitivity (97.7%), specificity (70.6%), PPV (91.0%), NPV (76.7%) and diagnostic accuracy was 84.0%. H-FABP demonstrated an Area Under Curve (0.911, 95%C. I:0.850-0.972, p<0.001), higher than that of hs-TnI (0.908, 95%C. I:0.844-0.971, p<0.001) in patients having chest pain for 3 hours or less, at the cost of specificity.

Conclusion: H-FABP (sensitivity 91.5%) is a more sensitive biomarker than hs-TnI (sensitivity 83.05%) in the early diagnosis of ACS, however being less specific, it can be used as adjuvant cardiac biomarker marker for early diagnosis of ACS.

Keywords: Diagnostic Accuracy, acute coronary syndrome; heart-type fatty acid binding protein; cardiac biomarkers.

Abstract-14

Evaluation of phosphorylated-tau as potential therapeutic target in Alzheimer's disease

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Introduction: Alzheimer's disease (AD) is the leading cause of dementia worldwide, and because of the aging world population its prevalence is still growing. About 6.2 million Americans of age 65 years and older are suffering from Alzheimer's dementia today and this number is estimated to grow to 13.8 million by 2060. The tau protein is a biomarker for neurofibrillary tangles (NFT) in AD and ensures microtubule stability, signalling pathways, and axonal transport. A change in tau structure leads to toxic accumulation. So, to reduce AD progression, the prevention of tau aggregation is a potential key factor to be investigated into, for drug discovery. We aim to highlight the presence of tau protein so that drugs can be developed to target this protein.

Methods: This was a basic science research performed in a lab setting at the Aga Khan University Karachi. Tissue homogenates of the cerebellum, pancreas and skeletal muscle tissue of mice, and cerebellum of mice of 3 different age groups (young, middle, old), were prepared. After protein quantification, SDS page and Western blot was run on the samples, followed by primary antibody incubation. Finally, an X-ray film was obtained.

Results: Our study confirmed the presence of tau protein bands in the tissue samples tested, at 55-62 kDa. They were found to be denser in the middle-aged cerebellum than young and old, as shown in the figure attached. Also, its presence was found to be less prominent in pancreas.

Conclusion: Research for future potential treatments of AD involves targeting the etiologic pathologies which also include p-tau. This is a step towards development of successful treatments that may stop or modify the course of AD.

Keywords: Alzheimer's disease, tau-protein, therapeutic target, mice.

Abstract-15

Evaluation of hormonal profile and Y chromosome microdeletion in azoospermic and severely oligozoospermic males presenting with primary infertility

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Introduction: Infertility is an emerging global health issue that affects millions of people globally, almost 15% of couples in both developing and developed countries during their reproductive age. Around 20-50% of these infertility cases are attributable to the male factor and 40-50% to female factor. Chromosomal aberrations (either numerical or structural) as Klinefelter syndrome, is the most common cause among genetic factors (in 30 % cases of male infertility). Y chromosome microdeletions are the second most frequent genetic cause with noted frequency ranging from 1 to 50%.

Methods: A total of 112 patients having primary male infertility (76 patients diagnosed with Azoospermia and 36 patients having severe oligozoospermia) were included. All the patients underwent detailed hormonal profile which included serum testosterone, FSH, LH and prolactin levels. Multiplex PCR was done for detection of Y-chromosome microdeletions. Total of eight STS markers including ZFX/ZFY and sex-determining region (SRY) on Yp arm used as the internal positive control as per recommendations of the European Molecular Genetics Quality Network (EQMN).

Results: Total of 3 (2.67%) cases of Y chromosome microdeletions were detected and all were observed in azoospermic males. All the three cases of microdeletions were involving AZFc region. No significant correlation was found between Y-chromosome microdeletions and levels of reproductive hormones.

Conclusion: AZFc microdeletions are the most common type of the deletion. AZFc microdeletions have better prognosis in couples opting for assisted reproductive techniques thus highlighting role of YCMD screening in non-obstructive azoospermic and severely oligozoospermic males.

Keywords: Y chromosome microdeletion, Azoospermia, Oligozoospermia, Reproductive hormones.

Abstract-16

Evaluation of apolipoprotein B / apolipoprotein A ratio as an alternate of lipid profile for cardiovascular risk assessment in a tertiary care hospital

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Introduction: Cardiovascular disease (CVD) is a major health concern globally. Dyslipidaemia including low-density lipoprotein cholesterol (LDL-C) is one of the crucial CVD risk factors. In this context, excessive number of Apo B-containing particles is a main trigger in the atherogenic process. Whereas Apo A comprising of high-density lipoprotein cholesterol (HDL-C) particles helps reducing the risk of atherosclerosis. The apo B/apo A ratio represents the balance between these particles. In this study, we evaluated of apo B/apo A ratio as an alternate to lipid profile for CVD risk assessment.

Methods: A cross-sectional, comparative study was done at Department of Chemical Pathology, AFIP, Rawalpindi in

collaboration with AFIC, Rawalpindi. Apo A and Apo B were analyzed on Roche Cobas C501 by turbidimetric technique and lipid profile was performed on Advia 1800 by spectrophotometry.

Results: A total of 300 individuals were enrolled in study and grouped in Group 1 and Group 2. Results of Group 1 cases showed LDL-C, Apo B/Apo A ratio, and Trop I(hs) 3.1± 1.2, 1.4± 0.8 and 1.68±0.32 respectively. While, Group 2 had LDL-C, Apo B/Apo A ratio, and Trop I(hs) of 1.9±0.5, 0.6±0.3, and 0.02±0.04 respectively. Statistical analysis was performed by using independent t-test and p value was <0.05.

Conclusion: Apo B/ Apo A ratio is better and earlier marker for cardiac risk assessment.

Keywords: CVD, Apolipoprotein A, Apolipoprotein B.

Abstract-17

Relation of three-point estimation of inflammatory markers with the outcome and severity of hospitalized patients of covid-19 infection in a tertiary care hospital of Peshawar

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Introduction: Since outbreak of Covid-19 infection very few studies are available about different time point estimation of inflammatory parameters which can predict outcome and severity of patients having Covid-19 infection in a better way. Present study was an attempt to address this issue.

Methods: A cross-sectional study of Covid-19 patients was conducted from February 2020 to August 2021 in Covid-19 ICU of RMI. A total of 116 adult subjects from both genders were included in study. Three-time point estimation of Covid-19 inflammatory markers was done for assessment of severity and outcome of disease. Statistical analysis was done by using SPSS 25.

Results: Out of 116 enrolled patients, 78(67.2%) were males, and 38(37.8%) were females, mean age was 62±13.39 years. On ROC curve analysis of inflammatory markers at admission, only LDH had acceptable AUC of 0.779. On univariable logistic regression all variables except gender, HS-CRP and D-dimer at admission significantly predicted mortality. On multivariable analysis after adjustment for covariates, old age, LDH, NLR, Diabetes mellitus (DM) and ischaemic heart disease (IHD) predicted the mortality. Three-time point estimation of inflammatory markers on basis of severity of disease revealed that median values of all markers at all three time points were higher in critically ill patients than severe, moderate, and mild ones.

Conclusion: Older age, NLR and LDH at admission, DM and IHD were associated significantly with mortality. On basis of disease severity, all markers at three time points were higher in critically ill patients.

Keywords: Covid-19, Lactate dehydrogenase, Logistic regression.

Abstract-18

Experience of participation in a congenital hypothyroidism screening programme

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Introduction: Congenital hypothyroidism (CH) is one of the most common Inherited Metabolic Diseases for which newborn screening is recommended universally. In many countries, newborn screening programme is run at a state level. However, such a wide-scale programme is lacking in our country. Our objective is to share the experience of participation in a provincial CH screening programme.

Methods: The study was performed between 13th July 2020 and 10th August 2021. Specimen for TSH was collected from the umbilical cords for neonates discharged during the first six hours of birth, and by heel prick for those discharged after 48 hours. Dried blood spot specimens were collected on filter paper cards and sent to the designated project laboratory.

Results: A total of 3620 babies were screened for neonatal TSH during this study. There were 1914 males and 1706 females. The median (IQR) TSH level of the available records was 3.3 (2.1-5.3) mIU/L. The TSH levels were greater than 20 mIU/L in 30 (0.82%) and between 15 and 20 mIU/L in 62 (1.71%) newborns. There was a median (IQR) turnaround time of 15(4-55) days for initial results. The documented recall rate for repeat testing was 0.87%. Out of the tests on which repeat results were available, discrepancy was observed in 92.86%. The reasons for delayed testing were logistical and IT related.

Conclusion: The administrative issues in CH screening programme need to be addressed for it to be successfully implemented in the region.

Keywords: Congenital Hypothyroidism, Newborn Screening Program, Inherited Metabolic Disease, Thyroid Stimulating Hormone.

Abstract-19

Clinical Spectrum of Vitamin D Deficiency: Frequency and clinical presentation of Vitamin D deficiency — A Cross-Sectional Study from a Tertiary Care Hospital, Karachi

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Background: Deficiency in vitamin D is a serious global public health issue. The aim of the study was to study the frequency, symptomatology, and clinical findings in patients with vitamin D deficiency. Moreover, we also studied the correlation between vitamin D deficiency and the prescribed drugs.

Methods: A cross-sectional study was conducted at the Chemical pathology section of the Department of Pathology, DUHS, and Dow Diagnostic Research and Reference Laboratory between [1-5-2022 to 30-6-2022]. A non-probability convenience sampling was used to recruit participants. All subjects were included in the study who presented to DDRRL for the evaluation of their Vitamin D levels. History was taken at the time of recruitment after taking informed written consent. A well-developed proforma was used to take a detailed history about sociodemographic, symptoms of vitamin D deficiency, radiological investigations, menopausal status, prescribed drugs, and supplementation. Patients with incomplete history were excluded. Vitamin D levels of 20 ng/ml or higher were considered normal for adults. Data entry and analysis were performed using SPSS. A chi-square test of association was used to find the correlation between vitamin D deficiency and clinical parameters.

Results: Among 106 participants the mean age was 39.55 ± 13.5 years. Thirty-six (34%) participants had mild vitamin D deficiency while 24 (22.6%) participants had a moderate deficiency. The study revealed that patients who were using anticonvulsants, antibiotics, or steroids were more likely to suffer from vitamin D deficiency (p<0.0001). Mild deficiency was detected in 51.9% of the population taking these medications, while 25.9% had a moderate deficiency.

Conclusion: A high number of participants were found to have mild and moderate vitamin D deficiency even though the majority were on vitamin D supplementation. We found a significant association between prescribed drugs like steroids and antibiotics with vitamin D deficiency. We recommend that physicians should screen the patients for vitamin D deficiency if they are taking the above-mentioned drugs for chronic diseases.

Keywords: Vitamin D deficiency, Clinical Spectrum, Prescribed supplements, Antibiotics, and steroids.

Abstract-20

Comparison of COVID-19 rapid antigen test results with PCR reactive/ non-reactive cases

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Introduction: Various diagnostic assays have been introduced during the early phase of this SARS-CoV-2 pandemic. In this scenario, many molecular tests and immunoassays were rapidly developed. But the validation, certification and application of these different diagnostic tests is highly recommended for using and suggesting them in various clinical labs or point of care testing (POCT).

The coronavirus disease shares many similar features with other diseases like seasonal influenza and viral pneumonia. However, its range of clinical symptoms and the characteristics of spread are somehow different. Presently, the standard method for diagnosing COVID-19 cases is considered to be the reverse-transcriptase polymerase chain reaction (RT-PCR). However, in order to perform RT-PCR assays, specific equipment's and services are required and this test is costly with high turnaround time. Hence for better screening and isolation of such patients a rapid antigen detection immunoassay (RAD) has been introduced and is particularly suitable for point-of-care testing (POCT).

The objective is to assess the diagnostic accuracy of Abbott PanbioTM COVID-19 Ag Rapid Test Device using RT-PCR as the reference assay, evaluating any false-positive/negative reactions to determine the specificity of this analytical assay.

Methods: The study design was Cross-Sectional analytical. This study comprised of 105 samples including both males and females. Nasopharyngeal specimens were already tested by reverse-transcriptase polymerase chain reaction (RT-PCR), then the same (nasopharyngeal) specimens were analyzed on Rapid Antigen Detection Test Device. Data was analyzed using SPSS 23.0 and EP evaluator.

Results: Out of 105 specimens, we got 88 PCR positive samples and 17 PCR negative which when compared with Abbott PanbioTM Rapid Antigen Test Device showed 60 positive and 45 negative samples having sensitivity of 75.86% (67.04-83.32% CI) and specificity of 100% (80.49-100%). The accuracy of the test was found to be 78.95%. This test was found easy to use and more appropriate for point-of-care settings.

Conclusion: This test has great potential to become a vital tool for the early diagnosis of SARS-CoV-2, particularly in situations with limited access to molecular methods. Therefore, it can be concluded that for people with a high clinical suspicion of COVID?19 and negative rapid test, RT?PCR test would still be considered a preferred option.

Keywords: COVID-19, RT-PCR, Rapid Antigen Test, Specificity, Sensitivity.

Abstract-21

Markers contributing to comorbidities in Autism

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Introduction: Autism spectrum disorder (ASD) is a neurodevelopmental disorder and there is prevalence of epilepsy in electroencephalography. This study evaluates the contribution of urinary biomarkers to comorbidities and clinical findings in ASD.

Methods: This exploratory study was carried out from August 2019 to June 2021 in Department of Pathology and Laboratory Medicine in collaboration with the Department of Paediatrics, Aga Khan University, Karachi. ASD children with and without epilepsy were included. Random midstream urine was collected from the children with ASD using Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DMS-V) diagnostic criteria. Urine organic acid profile (UOA) was evaluated using Gas Chromatography-Mass Spectrometry.

Results: The mean age of the study subjects (n=65) was 4.5±2.3 years. Parental consanguinity was noted in 31(47.7%) subjects. The mean age at the time of diagnosis of ASD was 2.3±1.5 years and 14(21.5%) suffered from epilepsy. Primary metabolic abnormalities occurred in 10(16.94%) cases of ASD who had history of seizures, most common being dicarboxylic aciduria. Using Chi Square analysis, statistical differences in hippuric acid and tartaric acid in ASD with and without epilepsy were noted with p value <0.05.

Conclusion: Urine organic acid profiling might be useful as potential biomarkers to predict comorbidities and risk of epilepsy in ASD. Further metabolomic studies are required to study the etiology and association of epilepsy and ASD.

Keywords: Autism spectrum disorder, epilepsy, Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DMS-V).

Abstract-22

Frequency of hypogonadism in beta thalassemia major patients receiving blood transfusion

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Introduction: Beta thalassemia major is a recessively inherited dyserythropoietic anaemia, due to defective synthesis of one or more globin subunits of the haemoglobin tetramer requiring blood transfusions. Human body does not have an internal mechanism to eliminate the surplus iron, thus iron overload and its sequelae follow, effecting growth, vital organs, sexual maturity, calcium metabolism and vulnerable endocrine functions. Most complication are due to iron deposition in pituitary gland. Multiple causes of hypogonadism but hypogonadic hypogonadism remains the commonest cause.

The objective is to determine the frequency of hypogonadism in beta thalassemia major patients receiving blood transfusion.

Methods: This cross-sectional descriptive study included 385 patients, regularly transfused at thalassemia center Bahawal Victora Hospital, Bahawalpur. Patients' data was recorded in questionnaires. Clinical characteristics relevant to growth and puberty were recorded. Serum FSH, LH, Testosterone assays were performed using Beckman Coulter Access 2 Chemiluminescence (CLIA) technique. Data was analysed by SPSS version 24. Means, standard deviation and frequencies were calculated for numerical variables.

Results: Hypogonadism was seen in 35.2% of the studied group. Delayed puberty was observed in 49.7% of studied group.

Conclusion: Delayed puberty and hypogonadism are obvious endocrinopathies showing higher frequencies in iron overloaded thalassemic patients. Regular endocrine evaluation and timely intervention can ensure sexual maturity and improved quality of life for these patients.

Keywords: Beta Thalassemia major, Hypogonadotropic Hypo-gonadism, puberty.

Abstract-23

Practices of vitamin D supplementation leading to vitamin D toxicity: Experience from a tertiary care hospital

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Introduction: The trend of prescribing Vitamin D (VD) preparations for nonspecific body aches and self-medication has increased significantly. The importance of vitamin D toxicity (VDT) has been underestimated. This study was done to determine the frequency of VDT (>150 ng/ml) in subjects and evaluate the VD supplements used by these subjects.

Methods: This descriptive cross-sectional study was conducted at the Section of Chemical Pathology, Aga Khan University Hospital Karachi from April 2020 to March 2021. Subjects with 25-hydroxy-vitamin D (25OHD) toxicity were contacted and information related to history of calcium and VD supplementation were collected. The statistical analysis was performed using the Microsoft Excel 2016.

Results: After satisfying exclusion criteria 186 subjects (78 were <18 years of age and 108 were adults) were included in final analysis. All of these were using VD supplements and the main indications were delayed growth/short height in 34(43.7%) paediatric, and aches or pains in 59(54.6%) adult subjects.

Most of the subjects, 138(74.1%) were taking oral supplements.Commonly prescribed preparation in adults and paediatric subjects was 200,000 IU in 76(70.4%) and 400 IU in 28(35.9%) respectively. Most subjects, 127(68.3%) took supplements for 1-3 months. Stated total supplementation ranged from 20,000 IU to 3600,000 IU in paediatric subjects and 200,000 IU to 96,00,000 IU in adults.

Conclusions: Supplementation is a leading cause of potential toxic levels of 25OHD. The condition can be prevented by careful use of VD supplements and consistent monitoring.

Keywords: Vitamin D supplementation, hypervitaminosis D, vitamin D, toxicity, deficiency, children, Pakistan.

Abstract-24

Impact of cyclosporine monitoring in allogenic haematopoietic stem cell transplant patients

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Introduction: Allogeneic haematopoietic stem cell transplant (allo-HSCT) is the most effective treatment option in haematological disorders. We saw the impact of cyclosporine (CsA) monitoring in allo-HSCT for various haematological disorders.

Methods: A five-year cross-sectional study, from 1stJan 2016 to 30th Dec 2020 was conducted at National institute of blood disease and bone marrow transplantation (NIBD) hospital laboratory, Karachi, Pakistan. Data of allo-HSCT patients till one year post transplant were collected.

Results: A total of 117 patients were included. The mean age was 16.3 ± 14.1 years with 73 (63.5%) males. Majority were of Beta thalassemia major present in 40(34.2.1%) and aplastic anaemia in 32(27.3.1%) patients. Post-transplant 12(11.2%) developed Graft versus host disease (GVHD) and of these five had acute GVHD, four had skin GVHD, two had liver related GVHD and one had gut related GVHD. Mean CsA trough level of GVHD patients was 283±117mg/dl versus non-GVHD 321±252mg/dl. Cyclosporine induced neurotoxicity (CIN) related symptoms were found in 14(12.1%). Confirmed CIN with positive posterior reversible encephalopathy syndrome (PRES) was noted in 6 (5.2%). Mean CsA level in CIN was 485.7±247.5 mg/dl and in non-CIN was 613.4±394.7mg/dl. Primary graft failure was found in 7(6.0%) and secondary graft failure was present in 4(3.4%) patients. The remaining patients achieved full engraftment with mean neutrophil and platelet counts. The engraftment days were 13±05,16±10 respectively. Ten (8.5%) patients expired within one year of post-transplant with CsA level 185±24mg/dl

Conclusion: Literature is scanty about the consensus guidelines for administration and monitoring of CsA in allo-HSCT patients. There is need of standardised protocol for immunosuppressive agents to prevent post-transplant complications and mortality.

Keywords: Cyclosporine, allogenic haematopoietic stem cell transplant, graft versus host disease, haematological disorders, cyclosporine induced neurotoxicity.

SCIENTIFIC SESSION ABSTRACTS (POSTERS)

Abstract-25

Persistence of anti-SARS-CoV-2 IgG among industrial workers

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Background: Persistence of SARS-Cov-2 antibodies over longer period of time is still debated. We assessed the persistence SARS-CoV-2 antibodies over a period of 06 months among healthy individuals of Karachi.

Methods: SARS-Cov-2-IgG antibodies of workers of Hilton Pharma Pvt Ltd were analysed by ELISA technique initially in June 2020 and then in September and November of the same year.

Results: Thirty-six male workers with mean age of 39±20 years were included. Fever and cough were most common symptoms (41.7%), weakness, allergy was less common. A significant association was noted of clinical symptoms with Covid PCR p< 0.0451. At baseline, (June 2020), 19 out of 36 tested positive for SARS-COV-2 IgG antibodies. Out of these 19, 08(42%) had symptomatic disease in previous months. Of the remaining 17, 7(41.1%) had borderline positivity at baseline and all were asymptomatic in previous months. The others were negative. Upon follow up testing, out of 19 seropositive, 17 remained positive in Sep 2020 with mean 38.8±29.8U/ml and 29.55±21.5U/ml in Nov 2020, among borderline positive five out of seven remained borderline positive in Sep 2020 with mean 10.3±5.49 U/ml, two were negative. A good correlation (r=0.403) was found between June-Sep antibody (p=0.013) while weak association was noted (r=0.024) in Sep-Nov (p= 0.88).

Conclusion: Persistence of Covid antibody by natural immunity response or external immunity production by vaccines still needs evidence. Studies with larger sample size followed over longer periods of time can help in this regard.

Keywords: Persistence, Anti SARS-CoV-2 IgG, industrial workers.

Abstract-26

Evaluation of daily post work shift experience of clinical chemistry laboratory professionals from a lower middle-income country

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Introduction: Laboratory professionals deal with various challenges and situations throughout their work shift and are coping to provide best possible outcomes promoting safety, care, and accurate diagnosis. This survey was undertaken to inspect the experience of lab personals at the end of their shift at multiple sub sections of a clinical chemistry laboratory.

Methods: The study utilized a cross-sectional survey design. The survey was administered online via google forms from medical laboratory professionals (n = 50) working at clinical chemistry, the Aga Khan University (AKU), Pakistan. A team comprising of chemical pathology consultant and senior medical technologist, designed the survey questionnaire. The responses were recorded on a scale from (1 to 5) from worst experience to best. Frequency and percentages were calculated for gender, age, and experience for different sub sections while descriptive results based on the responses were also recorded.

Results: The response was had from 32(64%) subjects. Best possible experience was acknowledged by 16(31.3%) and they were able to help other co-workers. The day was challenging while they have been able to handle the situation well was the response of 14(28.1%), 9(18.8%) thought they had a good day with lots of accomplishments, while 8(15.6%) were of the view that they had a challenging day and needed to rest till the next shift. Whereas the day was worse for 3(6.3%) laboratory professionals.

Conclusion: Following good laboratory practices the findings of this baseline survey would prove to be beneficial in designing future strategies to promote a healthy work environment.

Keywords: laboratory, professionals, survey, experience.

Abstract-27

Non-HDL Cholesterol as marker of cardiovascular disease in type-2 diabetes mellitus

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Objective: To determine the frequency of cardiovascular disease (CVD) and use of serum non-HDL-c as marker for therapeutic monitoring and prevention of CVD in Type 2 Diabetes patients.

Methods: A Cross-sectional study was conducted at the Medical Outpatient Department (OPD) and Chemical Pathology Department, Combined Military Hospital (CMH), Malir Cantt, Karachi from Aug 2021 to January 2022.

A total of 175 patients with Type 2 Diabetes mellitus (T2DM) were included in this study. After taking informed consent, the demographic and anthropometric data was recorded. Blood samples were collected and analyzed in the Chemical pathology laboratory for lipid profile, HbA1C, and Fasting Plasma Glucose (FPG). Non-High-Density Lipoprotein cholesterol (non-HDL-c) was calculated by subtracting HDL-c from total cholesterol. The patient identification was kept anonymous, and the data was used for research purposes only.

Results: The average age of the patients was 45.54±10.32 years. Among the 175 patients studied, the frequency of CVD in T2DM was observed in 86(49.14%) patients. Association of serum non-HDL-c levels and CVD in T2DM patients was significant. High rate of NHDL-c was significantly high in CVD positive cases as compared to CVD negative cases.

Conclusion: CVD and diabetes are amongst the commonest non-communicable diseases associated with major morbidity, mortality and burden on healthcare infrastructure. Data also highlights the need of screening for CVD risk in younger age group with newly diagnosed T2DM Public health programmes addressing risk factors for CVD that are amenable to intervention through lifestyle modification are needed. Non-HDL-c is considered as an excellent indictor of CVD in T2DM.

Keywords: Non-HDL-c level, Type 2 diabetic, cardiovascular diseases.

Abstract-28

PCR positivity and humoral response in Covid-19 natural infection

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Introduction: SARS-CoV-2 infection is a global pandemic with various clinical presentations ranging from asymptomatic to severe disease, eventually leading to death. With this varied clinical presentation, diagnosis mainly stays with laboratory findings. Reverse transcription-polymerase chain reaction (rRT-PCR)-based assays for detecting SARS-CoV-2 viral genome are in practice at present. It is considered that qualitative assays for corresponding antibodies are far from providing evidence of disease because of the varied immune response. Our study's objective is to investigate the degree of association between RT-PCR positivity and seroconversion after natural infection in the Multan City of Pakistan.

Methods: In this study, 219 Healthcare Workers (HCWs) with suspected SARS-CoV-2 infection were screened via Reverse Transcription Polymerase Chain Reaction for viral genome, followed by detection of corresponding antibody response in serum samples within 10 weeks of their first exposure against spike protein via Chemiluminescence immunoassay. Chi-Square tests were applied to evaluate the association of gender, age, antibody response and RT-PCR positivity for the SARS-CoV-2.

Results: It is evident from the results that there is a significant association between positive RT-PCR and detectable corresponding antibodies. However, we have found no evidence of an association between age and RT-PCR positivity and between age and detectable antibodies (P value >0.01). Furthermore, this study's results indicate that there is no association between gender and RT-PCR positivity and between gender and detectable antibodies (P value >0.01).

Conclusion: It is concluded that antibody detection against SARS CoV-2 virus spike protein is a useful laboratory tool for screening COVID-19 infection.

Keywords: COVID-19, PCR, Antibody test, Healthcare workers.

Abstract-29

Application of average of normal as statistical tool for monitoring of systemic error detection: A patient data-based quality control approach utilization in point of care testing

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Introduction: Average of Normals (AON) is a method of patient-based Quality Control. Its purpose is to detect shifts in the analytical process by monitoring trends in reported patient results. AON is most useful when data is collected regularly (daily), and for relatively high-volume tests (50+) samples per day. This study was undertaken to monitoring AON of blood glucose for early detection of analytical shifts and trends in POCT programme at the Aga Khan University hospital.

Methods: More than one million patient blood glucose results have been analyzed during the duration of three years. Result monitoring takes place on a daily basis with the help of EP evaluator (Data innovation).

Results: Average of normal from last six months is 130.6 with the average range of 122-138

Conclusion: Monitoring of AON are currently in use in our laboratory, and they rapidly detect SE, reducing the number of samples requiring correction and improving patient safety.

Keywords: POCT, AON, moving average.

Abstract-30

A retrospective study on thyroid testing ordering pattern

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Introduction: The thyroid gland is an endocrine gland, secretes thyroid hormones triiodothyronine (T3) and thyroxine (T4). Free-T3 (FT3) and Free-T4 (FT4) are their unbound forms which are unaffected by binding protein concentrations. TSH is recommended as the single most useful test in majority of thyroid disease patients, followed by FT4. There is very limited utility of FT3 testing except in cases of T3 thyrotoxicosis where FT3 is elevated with normal FT4. An increase in inappropriate requirement for FT3 testing has been observed with no significant impact on clinical diagnosis of the patient. In this study, we aimed to review retrospectively, the ordering frequency of various thyroid function tests and make recommendations to reduce inappropriate ordering advice of FT3 test.

Methods: A pilot study was done on thyroid function tests of patients of all ages presenting in Indus Hospital and Health Network, Karachi during the month of July 2022.

Results: There were a total of 2236 thyroid function tests ordered, out of which the frequency of TSH only was 72.4 %, followed by FT3 13.5%, FT4 8.5% and full profile of TSH, FT3 and FT4, 5.4%.

Conclusion: The most frequently requested test after TSH was FT3, reflecting that FT3 is still given preference by the clinicians for evaluation of thyroid disease. Thyroid profile test is an expensive investigation, and a systematic approach is needed while ordering. Therefore, ordering of FT3 should be limited to indications such as discordance between TSH and

FT4 levels or discordance between TSH and clinical symptoms.

Keywords: TSH, Free-T4, Free-T3.

Abstract-31

Performance of CSF kappa free light chains for the diagnosis of demyelinating disorders- A Comparison with oligoclonal bands detection via Isoelectric focusing coupled with immunoblotting

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Introduction: Isoelectro-focusing (IEF) to detect oligoclonal bands (OCB) in cerebrospinal fluid (CSF) is the gold standard approach for evaluating intrathecal immunoglobulin synthesis in multiple sclerosis (MS), but kappa free light chain (KFLC) is emerging as an alternative marker. The aim of this study is to test the accuracy for KFLC in detecting MS, using OCB via IEF as gold standard in Pakistani population.

Methods: Sixty-four patients were divided into groups of positive, negative, and matching, based on OCB via IEF. KFLC was performed for all patients and results were compared against OCB results. A KFLC cutoff of 1 mg/L was used to assign the patients as positive.

Results: There were no significant differences in age or gender between positive and negative patients. Positive patients showed significantly higher levels of KFLC compared to negative patients. The > 1 mg/L cutoff provided an overall error rate of 10.9%, with 87.9% sensitivity and 90.3% specificity. ROC curve analysis confirmed the high diagnostic performance of KFLC levels, with an AUC of 93.6% (95% CI 87.6% - 99.6%, p<0.001). The analytical time for KFLC is 3 hours and 30 minutes less than OCB via IEF. KFLC is also PKR 7,850 (65.4%) cheaper than the gold standard.

Conclusion: KFLC is a cheaper and time-saving alternative to OCB via IEF and should be performed prior to the contemporary testing. Implementing this will not only reduce laboratory burden but will serve as a cost-effective and time saving option for patients as well.

Keywords: CSF, OCB, IEF, immunoblotting.

Abstract-32

Indirect pancreatic function test- utility of faecal pancreatic elastase for diagnosis of exocrine pancreatic insufficiency in children

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Introduction: Testing of pancreatic function is a valuable tool in the assessment, diagnosis, overall management, and prognosis of pancreatic disease. The "gold standard," the direct pancreatic stimulation test is not widely used in clinical practice because of its complexity, cost, and invasiveness particularly in Paediatric population. Human pancreatic elastase 1 (FPE) has been suggested as an alternative faecal test of exocrine pancreatic insufficiency (EPI). This study was done to evaluate the frequency of EPI in Pakistani children based on FPE values at a clinical laboratory in Pakistan.

Methods: This cross-sectional study was performed at the section of Chemical Pathology, Aga Khan University Karachi from 2018 to 2021. A data mining of FPE results of age birth to 16 years was done from the laboratory information system. Duplicates were removed and only the first sample per patient was included, comprising of both inpatient and outpatients. FPE was analyzed using an enzyme-linked immunosorbent assay (Immunodiagnostic AG, Germany. Levels > 200 ?g/ml are considered normal value, 100 - 200 ?g/ml as slight to moderate EPI and < 100 ?g/ml suggestive of severe

EPI respectively). Data was analyzed using SPSS version 19.

Results: A total of 3324 FPE results were performed during the study period. After application of exclusion criteria, a total of 551 were included in the final analysis. There were 334 (60.6 %) males and 217 (39.4%) females. The median age was 206 (IQR0-730) days. The mean FPE levels were 167 (IQR 120-249) ug/ml. Slight to moderate and severe EPI was found in 85 (15.4%%) and 276 (50.1%) respectively.

Conclusion: A high frequency of EPI in children from a single center is alarming. There is need to advocate the utility of indirect pancreatic function tests for optimal screening and better health outcomes.

Keywords: FPE, EPI, exocrine pancreatic insufficiency.

Abstract-33

Investigating the quality of POCT device: evaluation of Quikread Go® HbA1c test with an IFCC traceable reference method at the central laboratory

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Introduction: Point of Care Testing (POCT) devices for measurement of glycated haemoglobin (HbA1c) is on the rise, it is essential to evaluate their performance in comparison to central laboratory. QuikRead go (Aidian Diagnostics) HbA1c assay is a portable and user friendly immunological in vitro diagnostic test for quantitative measurement of HbA1c from finger prick capillary blood or anticoagulated venous whole blood samples. This study was undertaken to compare the performance of QuikRead go with a central laboratory-based Siemens Advia centaur 1800 enzymatic method that is traceable to the IFCC reference method.

Methods: This cross-sectional study was performed at the section of chemical pathology, department of pathology and laboratory medicine, the Aga Khan University (AKU), Karachi, in May 2022. Method comparison was done between IFCC calibrated HbA1c Siemens Advia 1800 reference method and POC QuikRead go® according to CLSI EP09C-3rd edition. Data set was gathered from total of 20 venous whole blood samples for precision and method comparison study using EP Evaluator (Data Innovations).

Results: Precision study showed a coefficient of variation (CV) of 2.1%, within the total allowable error 9.0%. The observed Standard Deviation (SD) was 0.16, within the SD goal. On method comparison, the results were linear with slope 1.001 (0.926 to 1.077), intercept -0.45 (-1.03 to 0.13) and the correlation coefficient (R) was 0.9883 obtained via Deming regression.

Conclusion: The obtained results indicate that QuikRead go HbA1c showed comparable results with the HbA1c reference method and can be utilized for effective POCT.

Keywords: POCT, HbA1c, comparison.

Abstract-34

Organophosphate poisoning — A retrospective evaluation of serum cholinesterase levels at a clinical laboratory in Pakistan

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Introduction: Insecticides with organophosphate compounds (OPs) are widely used across the world. About 60% of all poisoning cases in Pakistan involve OPs, one of the most prevalent agents of poisoning. The cholinergic crisis results from suppression of the cholinesterase enzyme. Hence, serum cholinesterase levels can serve as an aid in establishing biochemical diagnosis. To assess the frequency, age, geographical location of patients with suspected OPs poisoning based on serum cholinesterase levels.

Methods: The descriptive retrospective study was conducted at the Section of Clinical Chemistry, Aga Khan University, Karachi. Serum cholinesterase results from May 2021 to June 2022 were retrieved from the lab information management system. Serum cholinesterase was analyzed by spectroscopy and instrument used for analysis is Micro lab 300. Statistical analysis was done by Microsoft Excel 2022

Results: A total of 254 Serum cholinesterase results were obtained, male (157) and females (97). Low cholinesterase levels were found in 38 males and 28 females suggestive of OPs poisoning. The majority of the cases were in 01-30, 31-50 years of age bracket being 51 and 11 respectively. All positive cases were from Karachi.

Conclusion: Frequency of OPs poisoning is alarmingly high from a single center. In resource limited set ups like where testing for RBC cholinesterase levels is not available, serum cholinesterase levels can serve as diagnostic tools for OPs poisoning.

Keywords: organophosphate, cholinesterase, Poisoning, Pakistan.

Abstract-35

Enlightening patient perception through education: A Pilot project on models of care for secondary fracture prevention

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Introduction: Robust patient education system tailored to local context and needs was designed for our patients to address the osteoporosis treatment gap. The objective of this study was to evaluate the educational impact of two different methodologies of delivering the information on the outcome of the consent rate for participation in Fracture Liaison Service.

Methods: From the inception of the study in November 2021 till March 2022, the research coordinator while taking consent from eligible patient/family members, provided simple descriptive verbal information regarding secondary fracture prevention and integrated models of care available in Pakistan. Whereas from April 2022 onwards visually descriptive resource pack was introduced in our native language (an intervention). Resource pack included four pictorial flyers that was provided to all patients in print form also to take with them. In this study we have determined the refusal rate for participation in the study pre and post intervention.

Results: The refusal rate pre-intervention was 41% (14 out of 34 eligible cases declined to provide consent to participate). The refusal rate post-intervention improved to 12% 2 out of 16 eligible cases to provide consent to participate (p-value <0.05) when the visually descriptive educational flyers were provided.

Conclusion: A well thought out, well-designed flyer designed according to local needs helped in convincing the patients to participate in a study that might improve patients' outcome. This highlights the importance of spending quality time and active engagement with patients to make them understand the importance of following care-path in preventing secondary fracture.

Keywords: osteoporosis, educational modalities, Fracture Liaison Service.

Abstract-36

Prevalence of cobalamin and folate insufficiency in a cohort of microcytic anaemia patients

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Introduction: Vitamin B12 and/or folate deficiency commonly result in macrocytosis. However, assessment of such deficiencies in cases of microcytic anaemia is also important due to overlapping mechanisms of genesis. Such an assessment could help in improving diagnosis in these patients. The objective is to determine vitamin B12 and folate deficiencies in patients of microcytic anaemia. The study design is Cross-sectional carried out between January and July 2021 at Department of Haematology, National Institute of Blood Disease, Karachi.

Methods: A total of 155 microcytic anaemia patients (Hb <13 g/dL for adult males or <11.5 g/dL for other participants with MCV <78fL) participated in the study. CBC was performed on Sysmex XN-1000 analyzer while vitamin B12 and RBC folate were assayed using Abbott "Architect" system. Data regarding effect modifiers like age, gender, socioeconomic status, area of residence and poor diet history was collected. The overall data was analyzed using SPSS version 23.

Results: Vitamin B12 deficiency (serum level<187pg/mL) and folate deficiency (RBC folate levels <126?ng/mL) were prevalent in 17.4% and 9% of the study sample, respectively. Cobalamin deficiency was particularly frequent among females in the age range of (32-46). No statistical significance was found in relation to effect modifiers and single nutrient deficiency.

Conclusion: Possibility of underlying cobalamin and/or folate deficiency should be considered in patients presenting with microcytic anaemia in the hospitals.

Keywords: Vitamin B12, microcytic anaemia, folate.

Abstract-37

Use of Sigma metrics to improve quality of total testing process

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Introduction: Sigma metrics is not only a mathematical tool, but rather a tool to monitor and improve quality of the whole testing process. Six sigma means an occurrence of six defects per million opportunities, which literally is World Class quality. The objective of the study was to improve the quality of our laboratory analytical quality by using six sigma tools.

Methods: The strategies used for six sigma analysis were Plan, do, check, act (PDCA) and DMAIC (define, measure, analyze improve, control). Sigma metrics were calculated for forty analytes performed on Alinity ci (Abbott Diagnostics) on three levels of quality control concentrations during January to December 2020. Analytes with sigma levels below three on all levels were identified. Root cause analysis was done on pre analytical, analytical and post analytical components of the testing process. Corrective actions were planned on the total testing process and implemented. Re-analysis was done the following year in 2020 to identify the progress. Steps to maintain and improve quality were defined.

Results: In 2020, there were nine analytes falling below three sigma on all three levels. After implementation of corrective actions, in 2021 there were only three parameters falling below three sigma. The main reasons identified for the sustained sub-optimal performance of these three assays were assay instability, practices and instrumental errors.

Conclusion: Six sigma is a quality improvement tool which requires a sustained and dedicated effort to be successfully implemented in the quality management process of clinical laboratories.

Keywords: DMAIC, sigma, testing process.

Abstract-38

Prevalence of vitamin D deficiency among children taking anti neoplastic therapy at a tertiary care hospital of Karachi

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Introduction: Children around the world are at great risk of developing vitamin D deficiency. A sufficient quantity of vitamin D is required for skeletal development and immune system and can aid in the prevention of several malignancies and autoimmune illnesses. Vitamin D insufficiency in childhood and adolescence can prevent optimal calcium absorption for skeletal growth. Children with cancer are more prone to vitamin D deficiency due to their pre-existing illnesses and the cancer treatments (alkylating agents and glucocorticoids) which may cause early bone loss, raising the risk of hip, vertebral and other fractures. The objective of the study is to evaluate the prevalence of vitamin D deficiency in children taking anti-neoplastic therapy and its impact on bone health.

Methods: This is the retrospective study conducted from November 2021 to July 2022. Serum Vitamin D levels of age group from 1 to 14 years presenting at the Oncology Department of Indus Hospital and Health Network on anti-neoplastic treatment was retrieved from EMR. The vitamin D levels were categorized as: <10ng/ml, 10- 25ng/ml, 25-80ng/ml and >80ng/ml. Data was analyzed using Microsoft Excel. Frequencies and percentages were calculated for the various categories of Vitamin D levels.

Results: There was a total of 65 patients, 36(54%) males and 29(46%) females. Overall, 36(56%) patients had Vitamin D levels below 10 ng/ml, 18(28%) between 10 to 25ng/ml and 10(16%) between 26 to 80ng/ml.

Conclusion: Children on chemotherapy are predominantly Vitamin D deficient due to their concurrent health issues, poor appetite and anti-neoplastic therapy side effects. Supplementation with Vitamin D is required to reduce the adverse effects of its deficiency.

Keywords: vitamin D, anti-neoplastic.

Abstract-39

Quality evaluation of immunoassay parameters by Six Sigma metrics (5th generation quality control)

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Introduction: In the clinical laboratory, an error can impose serious effect on patient's diagnosis and management. Six sigma is methodology applied to assess quality control of clinical laboratories and it measure defects per million, process outcome and this strategy combine bias, imprecision, and total allowable error which makes it a more reliable and effective way of quality evaluation. Sigma metrics consists of five stages that is better described by two models and defined as, analyse, measure, improve and control. The objective is to assess quality of immunoassay parameters by using six sigma metrics.

Methods: The study design was cross sectional. Internal quality control runs and is followed by external quality control. CLIA 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 3 subsequent months. If the sigma values are ?6, between 3 and 6, and <3, they are classified as good or unacceptable respectively.

Results: Six sigma values were found for TSH for both levels of IQC for 3 months. When the sigma values were analysed by calculating the mean of 3 months, folate, LH, FSH, Vitamin D, TSH and vitamin B12 were found >6. The mean sigma values of T3 and T4 were at sigma level 3.

Conclusion: When the analytical performance was evaluated according to Six-Sigma levels, it was generally found to be

good. It is possible to determine the test with high error probability by evaluating the fine sigma levels and the tests that must be guarded by a stringent quality control regime.

Keywords: Bias, Six sigma, key performance indicators, CV.

Abstract-40

Evaluation of key performance indicators in pre-analytical phase of testing in a clinical chemistry laboratory of a reference institute

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Introduction: Key performance indicators or quality indicators are useful tools for continuous improvement of laboratory performance and have a direct impact on the quality of patient care.

The study was Observational and Cross-sectional, conducted at Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from April to September 2021.

Methods: Defined key performance indicators (KPIs) were observed for a period of six months. Frequency and percentage of each KPIs were calculated. Defects per Million were calculated for deriving Six Sigma (σ) values. KPIs were also compared between morning and night shift using independent sample t-test and $p < 0.05$ was considered significant.

Results: A total of 272,731 samples were observed in which 2306 (0.84%) were found haemolyzed ($\sigma = 3.5$), 604 samples (0.22%) were not received in the department due to various pre-analytic reasons ($\sigma = 4.0$), 260 samples (0.09%) were found having insufficient sample volume for analysis ($\sigma = 4.5$), 181 (0.06%) samples were found having improper/ wrong labelling or bar code errors ($\sigma = 4.5$) and 161 (0.05 %) samples were delivered in wrong tubes ($\sigma = 4.5$).

Conclusion KPI-1, KPI-2 and KPI-3 were found significantly higher during the night shift as compared to the morning shift.

Keywords: KPIs, quality indicators.

Abstract-41

Oral manifestations and metabolic bone disease in children with transfusion-dependent beta-thalassemia major

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Introduction: Iron overload in transfusion-dependent children with ß-TM disrupts the bone homeostasis and altered vitamin D, calcium, and phosphate metabolism. Dental anomalies are common in patients and oral examination can reveal evidence of bone destruction. Dental and gingival health in association with bone disease of Pakistani children with thalassemia is unclear.

Methods: A multidisciplinary cross-sectional descriptive study was conducted at Fatimid Foundation and Aga Khan University after ERC approval. Data on clinical information, physical and oral examination was collected. Serum and plasma samples were analysed to test biochemical parameters of bone health. Dental and gingival health was assessed by calculating the decayed, missing, filled (dmf) index and visual modified gingival index (MGI).

Results: Serum analysis of 75 thalassemia patients aged 4 to 18 years revealed that all the participants had significant iron overload (mean serum ferritin= 3575.97, SD±2026.24). Low 25-OHD was present in 57(76%) patients, while low phosphate was present in 3(4%), high phosphate in 3(4%), low calcium in 15(20%)), low iPTH in 8(10.66%), high iPTH in 9(12%). The mean dmf score of the patients was 1.96±3.06. The mean MGI was 0.58±0.97.

Prominent maxillary bones and zygomatic bones were present in 17(22%) and 8(10%) patients respectively, while gingival pigmentation in 32(42%), enamel hypocalcification in 16(21%), crossbite in 14(18%), and mucosal pallor in 12(15%) patients.

Conclusion: The dental and gingival health of children with beta-thalassemia major is mildly affected in the presence of metabolic bone disease.

Keywords: thalassemia, metabolic bone disease, oral health status.

Abstract-42

Augmented artificial intelligence using collaborative laboratory integrated reports for aminoacidopathies reporting

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Introduction: Aminoacidopathies are complex and challenging conditions presents either with a grossly abnormal profile, while few having subtle deviations. The aim of the study was to interpret metabolic profiles of plasma amino acid (PAA) and compare reference intervals (RI) of PAA data from Pakistan with international laboratory using Collaborative Laboratory Integrated Reports (CLIR).

Methods: Twenty-two PAA profile in one year period were analyzed by cation exchange high performance chromatography at Biochemical Genetics Laboratory (BGL) of Aga Khan University Pakistan. The data was divided into two sets; reference and cases and uploaded on CLIR Software using Amino Acid in Plasma application for statistical analysis.

Results: Among total of 2081 PAA profiles, 92% were reported as normal with all PAA values falling within the age-specific reference range. The remaining one hundred sixty-eight samples had atypical profiles with 27.38% identified as non-fasting specimens with major aminoacidopathies identified were Phenylketonuria and Maple Syrup Urine Disorder.

Conclusion: A concordance of >90% was noted between the reporting done by the BGL team at our institute and after applying CLIR tools. Utilization of artificial intelligence like CLIR will improve accuracy of detecting inborn errors of metabolism including aminoacidopathies.

Keywords: Collaborative Laboratory Integrated Reports; aminoacidopathies; plasma amino acid; newborn screening.

Abstract-43

Correlation of serum erythropoietin levels with different stages of diabetic retinopathy

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Introduction: To determine the correlation of serum concentrations of erythropoietin with diabetic retinopathy in patients with type 2 Diabetes Mellitus. The study design id Cross-sectional and placed at Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from July 2021 to December 2021.

Methods: A total of 90 patients and 90 age-matched controls were included and studied. The 90 patients were divided into proliferative diabetic retinopathy (PDR) and non-proliferative diabetic retinopathy (NPDR) groups. The two subgroups were correlated according to a stage of PDR and NPDR for serum erythropoietin, Creatinine, HbA1c, and Hb. Independent-Sample Student t-test was applied between PDR and NPDR groups (p-value ? 0.05). Pearson's correlation was applied among disease severity, type of retinopathy, and EPO levels (p-value ? 0.05 was considered significant).

Results: The average age of participants in the control group and cases was 45.88±8.6 years and 56.6± 10.23 years, respectively. More males (n=60, 66.7%) were noted in cases compared to controls (n= 42, 46.7%). EPO concentration observed in cases (8.4151± 1.87 IU/L) was higher than controls (6.5053 ±0.91011 IU/L). Mean EPO concentration in PDR (9.35±1.74 IU/L) was greater than that in NPDR (7.338±1.384 IU/L). Among clinical stages, cases with severe disease

showed the highest concentration of EPO compared to mild and moderate cases (p-value <0.001).

Conclusion: Serum EPO concentrations increase in PDR compared to NPDR, and high EPO levels correlate with disease severity. Monitoring EPO levels can give clinicians an idea about the severity of DR and help them adopt better and timely treatment options.

Keywords: Erythropoietin; Diabetic retinopathy, proliferative diabetic retinopathy.

Abstract-44

Pattern of transfusion related zinc and copper derangements in beta thalassemia major patients

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Introduction: Beta thalassemia major is an inherited disease resulting from reduction or total lack of beta globin chains. Patients with this disease need repeated blood transfusion for survival. This may cause oxidative stress and tissue injury due to iron overload, altered antioxidant enzymes, and other essential trace element levels. Deficiency of Zinc in individuals with thalassemia can aggravate abnormal cellular function and oxidative stress leading to series of complications. Hence it is desirable to monitor zinc levels in these patients to prevent the harmful effects of its deficiency.

Methods: Serum Zinc (Zn) and copper (Cu) levels of all subjects were analysed on atomic absorption spectrophotometer. Kolmogorov Smirnov test was used to check for normality. Zn and Cu levels were expressed as mean ± standard deviation. Independent sample t-test was used to compare Zn and Cu concentration of patients with thalassemia major with that of healthy controls.

Results: Study included 80 subjects with mean age of 13.33 ± 7.69 years. The mean value of serum Zn and Cu in beta thalassemia major patients were 8.62 ± 1.77 µmol/L and 14.46 ± 5.92 µmol/L respectively as compared to 15.08 ± 2.8 µmol/L and 13.45 ± 2.80 µmol/L in healthy controls. Zn levels showed a statistically significant difference ($p = 0.005$) between two groups while Cu levels did not show any statistically significant difference between the two groups.

Conclusion: Beta thalassemia major patients showed significantly lower levels of serum Zn as compared to healthy controls which should be taken into consideration for continuous monitoring and prompt correction. Whereas no significant difference was seen in serum Cu levels among Beta thalassemia major patients and normal healthy controls.

Keywords: Atomic absorption spectrometry, Serum zinc, Serum copper, Beta thalassemia major.

Abstract-45

Biotin-responsive multiple carboxylase deficiency- Prevalence, clinical and biochemical characteristics in Pakistani population

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Introduction: Biotin responsive multiple carboxylase deficiency (MCD) is characterized by deficiency of a cofactor biotin, required by 4 carboxylase enzyme complexes; Acetyl CoA carboxylase, Propionyl CoA carboxylase, 3-methylcrotonyl CoA carboxylase and pyruvate CoA carboxylase. Aim of this study was to determine the clinical spectrum and biochemical findings on urine organic acids (UOA) in multiple carboxylase deficiency (MCD) patients presenting to biochemical genetics laboratory (BGL).

Methods: A cross-sectional study was performed at the BGL, AKU. Patients reported as MCD, from January 2013-December 2020 were included. The UOA were analyzed by gas chromatography mass spectrometer.

Diagnosis was based on peaks of 3-hydroxy isovaleric acid (3OHIVA), 3-hydroxy propionic acid (3OHPA), 3-methyl crotonyl

glycine (MCC), Tiglylglycine (Tig) and methyl citrate (MC) on UOA. Demographic, clinical, and biochemical details were extracted from BGL history form. Data was analyzed by Microsoft Excel 2010.

Results: Two hundred and two patients were reported to have biotin responsive MCD with 111(55%) males and a median (Q3-Q1) age of 7 months (13-4). Of these 145(71.7%) patients presented in infantile period. Parental consanguinity was observed in 161(80%) and another 66(32.6%) cases grandparents were cousins. The main presenting features were seizures, developmental delay, and lethargy. The common peaks were determined on UOA 3-hydroxy isovaleric acid (3OHIVA), 3-methyl crotonyl glycine (MCC), and methyl citrate (MC).

Conclusion: MCD is not rare in Pakistani population; it is recomended to include this disorder in newborn screening programmes.

Keywords: Biotin responsive multiple carboxylase deficiency, organic acids, Amino acids, Pakistan, inborn errors of metabolism.

Abstract-46

Anti-phospholipase A2 receptor (PLA2R) igg antibody test for the diagnosis of primary membranous nephropathy — performance evaluation

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Introduction: Membranous nephropathy (MN) is a common cause of nephrotic syndrome in adults. In 70% of patients with primary MN (pMN), the immune complexes consist of autoantibodies against podocyte protein M-type phospholipaseA2 receptor (PLA2R) which are rarely detected in secondary MN or other glomerulonephritis. We performed evaluation of PLA2R assay for the diagnosis of pMN against renal biopsy.

Methods: This cross-sectional study was conducted at Aga Khan University, Pakistan from March-November 2019. PLA2R testing was performed on ETI-Max 3000 immunoassay using Euroimmun test kit (Medizinische LabordiagnostikaAG). For analytical validation precision, accuracy, linearity and analytical measurement range (AMR) was assessed following CLSI guidelines.

Suspected samples of pMN with biopsy results were used for Alternate assessment by Deming regression analysis. Sensitivity, specificity, NPV and PPV were assessed. Concordance was calculated using Cohen's Kappa. SPSS version 21 and EP evaluator were used for statistical analysis.

Results: Precision study with level-1 and 2 controls were acceptable with mean±SD of 2.0522 ± 0.0682 RU/mL and 105.538 ± 2.210 RU/mL respectively.

Accuracy, AMR and linearity were analyzed by 5 calibrators over a measured range of 0.6-1500 RU/ml and found acceptable with slope of 1.024, intercept -0.002. Alternate assessment with peer lab revealed good correlation (r:0.99). Diagnostic accuracy(n=20) taking renal biopsy as gold standard yield sensitivity, specificity, NPV and PPV of 87.5 %,100%,92.3 % and 100% respectively. Concordance showed Cohen's Kappa of 89.4%.

Conclusion: Detection of the serum PLA2R antibody yield a high specificity and provides a non-invasive method for diagnosing pMN comparable to biopsy.

Keywords: PLA2R, pMN, renal biopsy.

Abstract-47

Characterization of a series of patients with cystathionine beta synthase deficiency

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Introduction: Cystathionine beta synthase (CBS) deficiency is recognized as the most common inborn error of sulfur amino acid metabolism, which metabolises homocysteine into cystathionine, leading then to the synthesis of cysteine. Aim was to characterise a series of patients with CBS deficiency presenting to a Biochemical Genetics Laboratory (BGL) in Pakistan.

Methods: A cross-sectional study was performed at the BGL, AKU. Plasma Amino acid (PAA) analysed at BGL by high performance liquid chromatography and Plasma homocysteine (tHcy) levels analysed by chemiluminescence immunoassay, from January 2013 to Dec 2019 were included. The CBS deficiency is diagnosed based on increased levels of Methionine, decreased cystine on PAA and high levels of plasma tHcy. Demographic, clinical, and biochemical details were extracted from BGL history form. Data was analysed by Microsoft Excel 2010.

Results: Over 7 years period 7259 patients were tested for PAA, of those 33 (0.4%) CBS deficiency was diagnosed in patients based on PAA, were included in the final analysis. The median (Q3-Q1) age of patients was 6.3 years (5-9), with the majority presenting after 4 years of age. The male to female ratio was 1:2. Parents of 22(67%) patients had consanguineous marriage. Median Plasma methionine, plasma homocysteine levels were 399 umol/L (568-83) and 190 umol/L (224.5-162.3) respectively.

Most common clinical feature was hypotonia/lethargy 9(27%), followed by seizures and developmental delay in 7(21%) and 18(54%), mental retardation 10(30%), eye lesions 5(15%),ectopia lentis 8(24 %) patients respectively.

Follow-up plasma tHcy levels were reduced to <50 umol/L in 10(30 %) patients only and average time taken this level was 126 days (325-65).

Conclusion: In the present case series one-fourth of the CBS patients presented with ectopia lentis while plasma tHcy levels were decreased to <50umol/l in one-third of the patients. In this context large scale awareness campaigns at both primary and tertiary care level and implement of newborn screening as public health policy are dire need of time.

Keywords: cystathionine beta synthase, newborn screening.

Abstract-48

Clinical, biochemical and radiological spectrum of glutaric aciduria Type-1: A single centre experience from Pakistan

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Introduction: Glutaric aciduria type 1 (GA-1) is caused by deficiency of glutaryl-CoA dehydrogenase. GA-1 is characterised by marked increase of glutaric acid (GA), 3-hydroxyglutaric acid (3-OH-GA) and Glutaconic acid in urine via gas chromatography/mass spectrometry (GC/MS). MRI shows widening of the Sylvian fissures, mesencephalic cistern and enlarged pretemporal subarachnoid spaces. Our objective was to describe the clinical, biochemical, and radiological features of Pakistani patients with GA-1.

Methods: This study was conducted at the Biochemical Genetics Laboratory (BGL) of The Aga Khan University Hospital (AKUH), Karachi. Urine organic acids (UOA) samples were analysed by GCMS. UOA chromatograms of cases diagnosed with GA-1 from 2013 to April 2022 were reviewed. Clinical details and MRI findings were recorded from the clinical history forms acquired with each test request.

Results: A total of 32 cases were reported over a duration of nine years in high-risk screening cases, 21(66%) were males.

41%, 37% and 22% from Sindh, Punjab, and KPK respectively. Consanguinity was seen in 66% cases. The median age of diagnosis was 270 (IQR: 207-330) days. MRI findings were suggestive of GA-1 in 22 (69%) cases while it was not available for 10(31%) cases. The most common clinical features were developmental delay (n=19), seizures (n=13), lethargy (n=12), hypotonia (n=10), poor sucking (n=6) and (n=4) 13% mental retardation. Cohen's kappa revealed an agreement of 100% for MRI and UOA.

Conclusion: Thirty-two cases of GA-1 over a nine-year period from a single centre, points towards high prevalence in Pakistan. Recognition of classical MRI finding followed by prompt UOA analysis can aid better clinical outcomes.

Keywords: Glutaric acid type 1 (GA-I), urine organic acids, GCMS, Pakistan, MRI.

Abstract-49

Urine orotic acid analytical validation on HPLC

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Introduction: Excretion of orotic acid (an intermediate in pyrimidine biosynthesis) in urine is increased in several urea cycle disorders and, in few disorders, involving the metabolism of arginine. The primary objective of this study was to validate quantification if urine orotic acid on high performance liquid chromatography (HPLC).

Methods: Following the College of American Pathologists standards of method validation analytical validation study was conducted at the Section of Chemical Pathology, Aga Khan University (AKU) in 2021. The principle of HPLC, by Agilent, was used to elute and quantify orotic acid from urine. An anion exchange column was applied to elute the orotic acid. It was then detected by ultraviolet detector; wavelength of 275nm. Using the ERNDIM Internal Quality Control System special assay for orotic acid in urine and proficiency test material following method optimization and validation studies were carried out: precision-study, accuracy, linearity, analytical measurement range (AMR), carry over and comparison of two methods on HPLC. The EP-Evaluator was used for statistical analysis.

Results: Precision study on ERNDIM urinary special assay (n=20) demonstrated a CV of 2.7% (QC-level one) and 3.6 % (QC-level two) with means 21.0 and 92.0 µmol/l respectively. Linearity study was also acceptable from 5-200 µmol/L; with ideal slope=1.0 and intercept=0.0. Linearity study was carried out on six standards that were analyzed in triplicate to verify AMR/linearity. Carryover study indicated no peak in-between injections and therefore endorsed accuracy of orotic acid measurements. On method comparison (n=50; proficiency testing samples) slope of 1.0, with -1.0 µmol/intercept L was noted. Since the initiation of this test in clinical service, January 2021 to March 2022, a total of 03 positive patients with high orotic acid levels suspected of having IMDs have been reported. Out of these, two had hereditary orotic aciduria with documented megaloblastic anemia.

Conclusion: Our team successfully validated a fast assay (forty-minutes) orotic acid quantification methodology using HPLC. The method is potentially able to discriminate diseased patients from reference subjects. Larger studies for clinical validation are required.

Keywords: Orotic acid, HPLC, validation, optimization, Pakistan.

Abstract-50

Cases of Primary Hyperoxalurias (PH) in high-risk patients screened at Biochemical Genetics Laboratory

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Introduction: Primary hyperoxalurias (PH) are devastating, autosomal recessive diseases causing renal stones. The exact prevalence of PH is unknown in Pakistan. It is a challenging disease for clinician and many of these patients with PH can be missed. Here we present our cases of PH identified in patients evaluated for urine organic acid (UOA) at Aga Khan University (AKU).

Methods: Samples of UOA were received from various cities of Pakistan for UOA testing from 2013--2022. The UOA analysis was done on GCMS, Agilent.

Results: A total of 14131 urine samples were run on GCMS from 2013 to 2022. A total of eighteen PH cases were noted in this duration.; 7 from Sindh, 7 from Punjab, and 4 were reported from KPK. Median age of PH was 90 days; M:F ratio of 3:1. Two patients gave clinical history of renal stones in family, three had history of fits, one had sibling death and twelve did not have any significant history. Out of the total eighteen, twelve had consanguineous marriages.

Study of chromatograms of UOA analysis showed eight patients with peaks of oxalic, and glycolic indicative of PH1, three patients had significant peak of glyceric acid indicative of glyceric aciduria, while five patients were identified as having primary hyperoxaluria, two had clear peaks of oxalic and glyceric acid demonstrating PH2 (both were siblings with clinical history of recurrent renal stones).

Conclusion: There is a need to spread awareness of the clinical utility of UOA amongst clinicians in Pakistan to help accurate timely diagnosis of patients suffering from PH.

Keywords: metabolic defects, primary hyperoxaluria, pediatric, urine organic acids.

Abstract-51

Comparison of serum sodium level measured by Direct and Indirect ion selective electrode in critically ill patients with hypoalbuminaemia

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Introduction: Serum electrolytes are one of the most frequently requested tests in patients from emergency department or critical care settings. There are two methods for estimation of serum electrolytes, direct and indirect ion selective electrode (ISE). In Direct Ion selective electrode, the activity of the electrolytes is measured in plasma without dilution while in case of indirect ion selective electrode method, there is a pre-analytical serum or plasma dilution step which can result in pseudonormonatraemia or pseudohypernatraemia due to electrolyte exclusion effect. Discrepancy in sodium results can occur between two methods in case of hypoalbuminaemia which can lead to the misdiagnosis and mismanagement of critically ill patients. The objective of the study is to compare the mean serum sodium level measured by direct and indirect ion selective electrode in critically ill patients with hypoalbuminaemia.

Methods: The cross-sectional study conducted in department of Chemical Pathology, Sheikh Zayed Hospital, Rahim Yar Khan from January 1, 2022, to August 31, 2022. A total of 102 patient samples satisfying the inclusion criteria were included in the study. Analysis of included samples were performed for serum sodium level by direct and indirect ISE methods. Mean serum sodium level were measured by both methods compared and the difference was calculated. Serum cholesterol and triglyceride were also estimated in study samples. Data was recorded for age, gender, residence, duration of illness, duration of hospital stay, chronic liver disease, chronic kidney disease, congestive cardiac failure, malnutrition and inflammatory condition. P value <0.05 was taken as significant.

Results: Mean difference of serum sodium level measured by direct and indirect ISE (Indirect ISE-direct ISE) was 4.216±13.571mmol/L with statistically significant difference (p value: 0.031). Effect of triglyceride was statistically significant for mean difference of serum sodium level (p value: 0.029) No statistically significant difference of mean difference of serum sodium level was observed with respect to age, gender, residence, serum cholesterol, serum albumin, duration of illness, duration of hospital stays, chronic liver disease, chronic kidney disease, inflammatory condition and malnutrition.

Conclusion: Interchangeable use of direct and indirect ion selective electrode methods should not be used, especially in the setting of hypoalbuminaemia. Indirect ion selective electrode results in overestimation of serum sodium in the case of hypoalbuminaemia which results in misclassification and misdiagnosis of pseudo normonatraemia and pseudo hypernatraemia. Direct ion selective electrode method should be used preferably in setting of hypoalbuminaemia.

Keywords: Sodium, Direct ISE, Indirect ISE, Hypoalbuminemia.

Abstract-52

Diagnostic performance of SARS-CoV-2 rapid antigen test among symptomatic and asymptomatic patients in emergency department of a tertiary care hospital

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Introduction: Accurate and rapid detection of the SARS-CoV-2 is key for both timely treatment and controlling the community spread of COVID-19. Thus, this study aims to establish rapid antigen tests as an effective diagnostic tool to improve the testing strategies of COVID-19 diagnosis.

Methods: Two parallel nasopharyngeal swabs were collected from 719 consecutive patients admitted through ED. SARS-CoV-2 Rapid Antigen Test (Roche) was performed followed by SARS-CoV-2 real-time polymerase chain reaction (RT-PCR). Diagnostic performance of RAT was presented in terms of sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy and area under the curve (AUC), keeping SARS-CoV-2 RT-PCR as gold standard.

Results: The mean age was 46.03±17.74 years (range 18 to 86 years), 378 (52.6%) were males. Prevalence of SARS-CoV-2 was found to be 13.1% as diagnosed by RT-PCR. The overall sensitivity of the RAT was 98.61%, specificity was 99.52% and diagnostic accuracy of RAT was 98.61%. The sensitivity of the RAT was higher in symptomatic patients 95.18%, while RAT was found to be more specific in asymptomatic patients with a specificity of 99.83%. High diagnostic accuracy of 91.81% and 96.29% was noted in symptomatic and asymptomatic patients respectively. Area under the curve (AUC) for RAT was 0.93 and 0.86 in symptomatic and asymptomatic individuals respectively.

Conclusion: Our study concludes that SARS-CoV-2 RAT has high diagnostic accuracy in symptomatic and asymptomatic patients. It has shown great potential as a vital diagnostic tool for identification of SARS-CoV-2 patients in busy emergency departments or in situations with limited access to sophisticated molecular methods.

Keywords: SARS-CoV-2 Rapid Antigen Test, Diagnostic Performance, SARS-CoV-2 real-time polymerase chain reaction (RT-PCR), COVID-19.

Abstract-53

Cultivating Teaching-Learning Through Online Course on Bone Disorders Using a Multidisciplinary Multi-Institute Approach

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Introduction: The pedagogy of interdisciplinary teaching promises a range of desirable educational outcomes for students. An online course on osteoporosis was designed using experts from multiple disciplines and institutes and its educational impact on knowledge gain and experience by post-graduate trainees (PGs) and facilitators was evaluated.

Methods: A multi-disciplinary, multi-institute faculty team was formulated with experts from radiology, orthopedics, medicine, endocrinology, clinical chemistry, and education from across Pakistan to deliver a course on osteoporosis on virtual learning environment (VLE). Course content consisted of recorded micro-lectures, flash cards, case challenges, and mini-interview with the experts. PGs from various disciplines and institutes were enrolled after taking informed consent. Educational impact was measured by pre, and post-tests and feedback of the course execution was taken on a Likert scale of 1-5.

Results: The course was piloted on PGs (n=9) from various health institutes of Pakistan. Pre-test was attempted by 6(66%) PGs with a mean score of 43.8%. All PGs (n=9) cleared the end of module test with average score of 96%. All PGs 9(100%), believed that VLE is a great platform for online courses and were satisfied by this teaching strategy. Majority, 88.9%, stated that they could easily navigate the course using VLE and agreed that course learning was implementable in clinical practice, whereas 6(66.7%) were extremely satisfied with learning objectives and the content uploaded. The process also led to capacity building of facilitators who became comfortable using VLE.

Conclusion: An institute with resources for faculty training can develop online multidisciplinary modules for the benefit of other institutes with resource limited settings.

Keywords: Online course, Bone disorders, multidisciplinary multi-institute approach, educational impact, and faculty development.

Abstract-54

Determination of variation in neonatal serum 17 - hydroxyprogesterone levels in relation with gestational age and low birth weight

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Introduction: Screening newborns for congenital adrenal hyperplasia (CAH) is problematic owing to the dynamic changes in serum 17-hydroxyprogesterone (17-OHP) following birth. Serum 17-hydroxyprogesterone levels are falsely increased in prematurity and low birth weight. The objective is to determine the variation in neonatal Serum 17 - hydroxyprogesterone levels in newborns according to gestational age and birth weight.

Methods: It was an analytical cross-sectional study conducted at Armed Forces Institute of Pathology. The data were divided into following three groups.

Group I: Control group, Full term 38-40 weeks of gestation with normal birth weight 2500-4000g

Group II: Preterm i.e., 28 - 37 weeks of gestation.

Group III: Low birth weight i.e., < 2500 g.

Results: It was determined that Serum (17-OHP) levels were falsely elevated in 60 % cases of prematurity. Whereas in low-birth-weight infants, levels of Serum (17-OHP) were increased in 45 % of cases.

Conclusion: False-positive newborn-screening rates are disproportionately increased in prematurity and low birth weight. The optimal cut off levels of Serum (17-OHP) should be established for screening of patient of CAH.

Keywords: CAH, neonatal, 17-hydroxyprogesterone.

Abstract-55

Intra-familial Screening for Helicobacter Pylori Infection using the Urea Breath Test

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Introduction: Helicobacter pylori (H. pylori) infection spread is more common in low socioeconomic status and Intrafamilial spread. The aim of this study is to evaluate the intrafamilial acquisition of H. pylori organisms by investigating the urea breath test of household members of an index case with H. pylori infection.

Methods: A cross-sectional study was conducted from January to June 2022. The index cases with positive H. pylori infection were identified and their immediate household members, persons living with index cases for >3 months, voluntarily participated in the study and underwent a 13C urea breath test. For comparison three negative persons and their household members were included. Analysis of the breath samples was performed by UBiT- IR300 Infrared Spectrophotometer of POCone Infrared Spectrophotometer.

Results: Families of 11 index cases and 3 controls were included, total participants were 63, 49 in group I (index cases + their household members) and 14 in group 2 (controls + their household members).

In group, I, 33(67%) were positive on UBT testing while 6(43%) were positive in group II. The odds for a household member of an index case to have positive UBT or developing H. pylori infection was 2.4, while the odds for a household member of control was 0.75.

Conclusion: The spread of infection is higher in households of a person with an active H. pylori infection. Therefore, the eradication strategies should be focused on the family rather than a single person.

Keywords: Intrafamilial screening, Infections, Helicobacter pylori.

Abstract-56

Piloting models of care for secondary fracture prevention-interventions to capture hip fractures at tertiary care centre in Pakistan

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Introduction: Case identification for secondary fracture prevention is the backbone of fracture liaison service. This study was done to determine the gaps in case identification for inclusion and to propose solutions applicable to our hospital system.

Methods: Multidisciplinary team including bone specialist, chemical pathologists, orthopaedics & family medicine consultant and rheumatologist utilized "Plan Do Check Act (PDCA) cycle" to identify gaps and propose solutions to ensure optimal identification. Total cases included in the month of November, 2021 were analyzed with record from admission office. Gap analysis was performed to identify potential cases not reported in the admission data. Based on the admission data, nine patients were identified for inclusion in the different models of care. Whereas review of Operation-theatre records revealed that fifty-four surgeries of hip-fractures was performed. Team proposed that research coordinator should have Critical Care Management (CCM) access to timely identify cases admitted under various subspecialities. The case inclusion data and hospital admission record statistics were compared in the subsequent team meeting to evaluate the effectiveness of the proposed solution.

Results: Results after gap analysis showed improvement and nineteen additional patients were identified through CCM which were not included in admission data. Provide a summary and discussion of the results.

Conclusion: Team and research coordinator followed up the identified problem and effective implementation of the proposed solution which led to a standardized protocol for optimal patient identification. Moreover, any new or persistent problems will be brought into the next round of the PDCA cycle for further improvement Statement of the study's conclusions and/or implication of the results.

Keywords: Osteoporosis, Fracture Liaison Service, Multidisciplinary team.

Abstract-57

Determining Mutations in Biotinidase Gene Using Sanger Sequencing

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Introduction: Biotinidase deficiency (BTD) is caused by mutations in the Biotinidase gene, located on chromosome 3p25. More than 150 mutations in the gene cause BTD, of which majority are homozygous or compound heterozygous. New mutations are continuously being added to the growing list of mutations in Biotinidase gene that are helping to delineate structure/function relationships of the enzyme if they are not treated with biotin in adequate dosages. Since the treatment is simple and widely available, the disorder is also included in newborn screening. To optimize and validate Biotinidase gene analysis using Sanger sequencing.

Methods: A descriptive, cross-sectional study based on a convenience sampling strategy utilizing previously diagnosed case, was performed at Sections of Chemical Pathology & Molecular Pathology, Department of Pathology and Laboratory Medicine, AKU.

Whole blood samples were acquired for genomic analysis from previously reported and clinical confirmed cases of BTD on urine organic acid (UOA) analysis. Sanger Sequencing of Biotinidase gene was performed. The variant sequences were described according to the HGVS nomenclature system (http://varnomen.hgvs.org/).

Results: A total of 11 samples were analysed by Sanger sequencing including two samples from parents of a diseased child. We identified homozygous pathogenic mutations p.Arg518Ser in 4 cases in exon 2, c.1552C>A in 2 cases and c.38_44delInsTCC in 1 cases in exon 4. The parents were identified to have heterozygous mutations of c.38_44delInsTCC. Mutations were not identified in 2 cases.

Conclusion: We optimized mutation detection for BTD by sanger sequencing.

Keywords: Biotinidase deficiency, sanger sequencing, optimization.

Abstract-58

Innovative Assessment Tools in Chemical pathology: A Journey of Objectivity

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Introduction: Examiners and faculty of Chemical Pathology continuously expressed the need of improving the assessment tools of various examination. We conducted this study to determine the innovative assessment tools for summative assessment of postgraduate trainees of Chemical Pathology.

Methods: An exploratory study was conducted for which a team was formed which included Chemical Pathology faculty working in different academic centres across Pakistan. After thorough deliberations, some new examination tools were developed and tested in short intensive training courses and mock examinations. This process spanned over a decade before these tools were finally introduced in summative examinations of the College of Physicians and Surgeons Pakistan.

Results: The following assessment tools were introduced in summative examination for the Chemical Pathology fellowship programme:

Multiple Choice Questions (MCQs): A pool of about 2000 reviewed One Best Type MCQs was developed for the objective assessment of clinical and non-clinical topics of Chemical Pathology. The real success was creation of scenario-based C3 type of MCQs for lab-oriented areas.

Quick Assessment of Data Interpretation Skills (QADIS): This tool tests data interpretation skills, so that the future specialists develop a reflex to interpret data on an inquiry from a clinical colleague or a patient. QADIS is a modification of previously used examination module of data interpretation with more objectivity and standardization

Structural Assessment of Analytical Skills (SAAS): Analytical Skills Assessment has been made objective by adding novel statistical methods in the exam. This tool now focuses analytical skills very objectively and effectively excluding cognitive part.

Task Oriented Assessment of Clinical Skills (TOACS): This examination is the CPSP version of OSCE and OSPE and widely used in clinical examinations. The real challenge was development of TOACS stations for non-clinical topics. Collective wisdom of faculty of Chemical Pathology met this challenge very effectively and TOACS is now used for the assessment of IMM and FCPS Part II.

Method Evaluation Assessment Tool (MEAT): This tool is still under evaluation and will be used for the assessment of method validation (or evaluation) skills.

Conclusion: These assessment tools improved the objectivity, standardization and validity of the fellowship summative examination. The greatest innovation is inclusion of novel statistical methods.

Keywords: Assessment Tools; Psychomotor Skills; Chemical Pathology.