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41st Annual Conference of Pakistan Association of Pathologists

&

6th Joint International Conference of Societies of Pathology

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WELCOME MESSAGE BY THE CONFERENCE CHAIRMAN

Dear Colleagues,

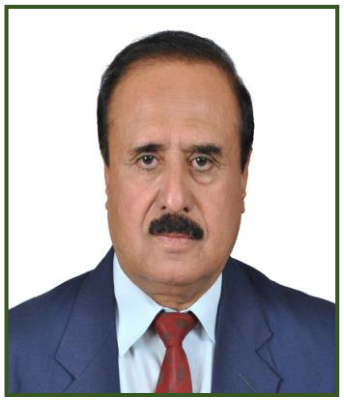
With great pleasure I would like to cordially welcome you to **41st Annual Conference of Pakistan Association of Pathologists and 6th International Joint Conference of Societies of Pathology Pakistan, 2018.** On behalf of Pakistan Association of Pathologists and the organizing committees at Armed Forces institute of Pathology Rawalpindi Pakistan I greet all the participants from Pakistan and abroad. Various preconference workshops are planned from 6th November 2018 to 8th November 2018, at Armed Forces institute of pathology, Armed Forces Institute of Transfusion Medicine, and Armed Forces Institute of Bone marrow transplant and National Institute of Bone marrow Transplant Rawalpindi Pakistan. Experts from abroad are participating to provide hands on experience to attendees. Communication of up-to-date information in the field of molecular pathology relevant to all the disciplines of pathology and precision medicine is the aim of present conference. Main theme of the conference is, Advances in Molecular Pathology – insight into precision medicine. Conference, Exhibition by renowned firms in the field of diagnostic laboratories and health care systems and scientific presentations are organized at Pearl Continental Hotel Rawalpindi Pakistan on 9-10 November 2018. The help of Army Medical College Rawalpindi, AFBMTC-NIBT, AFIT and National institute of Health Islamabad is very much gratifying and highly appreciated. The scientific rigor and desirable participation by the societies of Pathology is admirable to come to the common forum for all the Pathologists. This will generate and foster culture of collaborative research and development in the field of pathology.

I would also like to invite the honorable participants to enjoy and experience the twin cities of Rawalpindi and Islamabad. On 11th November trip to Bhurban Golf club and Brunch will be a feast to enjoy. The entire participants who register well in time for the excursion are welcome to this sheer relaxation in green entertaining environment of Murree Hills.

We, AFIP team look forward to a very successful conference and meeting you all.

Maj Gen
Muhammad Tahir Khadim
Advisor in Pathology/
Commandant AFIP,
Dean Faculty of Pathology
National University of
Medical Sciences (NUMS)
Editor-in-Chief

MESSAGE BY PRESIDENT PAKISTAN ASSOCIATION OF PATHOLOGISTS



Professor
Dr Ghulam Sarwar Pirkani
President
Pakistan Association of Pathologists (PAP)

It is a matter of immense personal pleasure and feeling of achievement, due to the fact that I am a member of the Journal of Pathology, the Pakistan Association of Pathology, all achievements lend a level of genuine personal and professional satisfaction.

It is indeed a highly fundamental achievement that the Pakistan Journal of Pathology has published this issue on the milestone event of the joint conference of the 41st Annual Conference of PAP and the 6th of the Pakistan Society of Pathology, which also marks that the various sections and units of PAP are now fully functional, and playing their invisible but vital and fundamental role in the development of the field of Pathology in Pakistan.

Here, it is highly pertinent to note and deeply recognize and appreciate the labor of love and the conscientious efforts which are glaring visible of the Editorial Board of the Journal of Pathology with their team, in ensuring that the Journal is published regularly and has now reached the present stage of repute. The place and value of this journal for the development of Pathology and the Pathologist in the country cannot ever be overemphasized. The journal has established itself as a reputable and serious scientific forum in a pivotal position for the development of the field of Pathology and communicating authentic research in Pakistan. The benefits of the Journal are not limited to the Pathologist, its spring of knowledge is not limited by rather transcends professional boundaries, where all who seek knowledge and skill are equally gaining benefits from this avenue of knowledge and skills to serve humanity, inside as well outside Pakistan, knowledge has no boundaries.

I once again congratulate the Editorial Team of the Journal and their support team for this remarkable achievement, and wish that they and their future team members will continue with this labor of love, knowledge production, dissemination, skill development, and professional accomplishment in service to people and the profession in and outside the country.

EDITORIAL

With great pleasure I welcome you to the special abstract supplement of Pakistan Journal of Pathology, an official journal of Pakistan association of Pathologists. All the abstracts submitted for the 41st Annual conference of Pakistan Association of Pathologists and 6th international joint conference of societies of pathology are included in this volume. Articles not accepted for oral presentation will be presented in poster format at the conference venue. Each abstract has been assigned identification number for archive and retrieval for reference. The articles have been grouped according to various disciplines of Pathology. The 41st Annual conference of Pakistan Association of Pathologists and 6th international joint conference of societies of pathology will be held at Armed Forces Institute of Pathology (AFIP) and Pearl continental Hotel Rawalpindi Pakistan on November 9 – 10, 2018. Preconference workshops are planned from 6th November to 8th November 2018. A total of 18 workshops will provide rich opportunities for the participants to get hands on experience on NGS and other advances in the field of molecular pathology in the context of various disciplines of pathology. The main theme of 2018 conference is “Advances in molecular pathology- an insight into precision medicine”.

Pakistan Association of pathologists provides a common platform for all the disciplines of pathology to collaborate, interact and develop the culture of collaborative research. During the annual conference renowned scholars will present and share their experiences with the colleagues and students. This year the conference will provide a unique opportunity for authors and participant to network and interact with the experts in relevant fields. The idea of this conference is to further foster the culture of learning, research and collaboration among all the disciplines of Pathology in Pakistan.

The team at AFIP Rawalpindi look forward to enthusiastic participation by all the pathologists. We wish all the success to societies of Pathology during the conference to achieve their professional goals. We invite and welcome junior pathologists from all over the country to join the Pakistan Association of pathologists.

Major General
Muhammad Tahir Khadim
Advisor in Pathology/ Commandant AFIP,
Dean Faculty of Pathology
National University of Medical Sciences (NUMS)
Editor-in-Chief

PLENARY SESSION LECTURE ABSTRACTS

PS-Lec-0001

RAZI LECURE:

PATHOLOGY IN PAKISTAN – PAST, PRESENT AND FUTURE

Lt Gen Manzoor Ahmed HI(M)(Retd)
MBBS, FCPS, FRCP, (Edin), FRC Path
Diplomate American Board of Pathology

PS-Lec-0002

JABBIR IBN HAYYAN LECTURE

MAMMAPRINT 70-GENE BREAST CANCER RECURRENCE ASSAY

Dr. Saira Alvi
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ABSTRACT

"MammaPrint" is a genomic test to predict risk of breast cancer recurrence. It analyses the 70 most important genes associated with breast cancer recurrence and classifies patients into "Low Risk" and "High Risk" of developing metastases within the first 10 years after diagnosis. "BluePrint" is a test to predict tumor behavior in breast cancer. It analyses the activity of 80 genes to stratify tumor into three subtypes: Luminal-type, HER2-type and Basal-type.

MammaPrint and BluePrint can be run at the same time on the same tissue sample to provide results in ten days. This is the only FDA cleared genomic breast cancer test recommended in guidelines by NCCN and the American Society of Clinical Oncology, that is designed for women of all ages who are:

- Who are newly diagnosed with invasive early-stage breast cancer (Stage I or II)
- Have a tumor size up to 5 cm
- Are either lymph node-negative or have 1-3 positive lymph nodes

In MINDACT clinical MammaPrint trial of 6,693 early stage patients, 46% were spared chemotherapy. MammaPrint analyzes 70, and BluePrint analyzes 80, of the most important genes associated with breast cancer to help oncologist make chemotherapy treatment decisions. "MammaPrint" and "BluePrint" in combination enables the Oncologist to make the best treatment decision for the breast cancer patient.

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PS-Lec-0003

A VIRAL DETECTIVE STORY BEFORE MOLECULAR PATHOLOGY

Dr David R Franz
DVM, Ph.D
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ABSTRACT

In October of 1989, a senior virologist at the U.S. Army Medical Research Institute of Infectious Diseases received a phone call from the veterinarian responsible for a large industrial research primate colony in Reston, Virginia. Some of the animals in a shipment of 100 *Macaca fascicularis*---the crab eating macaque---which had recently arrived from near Manila in the Philippines were dying. Simian Hemorrhagic Fever was the presumptive diagnosis, a viral disease that does not affect humans…but something just wasn't right. Eventually, the colony managers provided tissues from one dead monkey and the detective work began. Dr. Franz will describe the technical approaches, the human factors and the politics surrounding the unexpected chain of events that followed. Soldier-scientists from a maximum-containment army laboratory would respond to a potential viral crisis in a civilian community only 20 miles from the center of Washington D.C.

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PS-Lec-0004

THE EMERGING ROLE OF DNA ANALYSIS AND PRECISION MEDICINE – PERSPECTIVE FROM ISLAMIC BIOETHICS

Dr Marcia Hermansen
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ABSTRACT

This presentation will provide an overview of bioethical issues raised by genomic testing, specifically recent advances in precision medicine in which new generation sequencing (NGS) of DNA and RNA is used in assessing the best treatment options for patients with a range of cancers. Genomic testing is now being used to find out what is driving cancer in an individual patient. It may then be possible to identify treatments that target the specific driver of the cancer for that patient instead of attacking all, even healthy, cells.

The paper will consider bioethical issues of such testing

that are general such as 1) Access 2) Privacy 3) Expectations 4) Regulation and 5) Research Potential, while indicating culturally specific dimensions of these along with other ethical sources to be considered when making such testing available to Pakistanis, for example, regional and Islamic contexts.

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PS-Lec- 0005

MOLECULAR PATHOLOGY

Dr. Rizwan C Naeem
Prof of Pathology, Albert Einstein College of Medicine & Montefiore Medical Center, United States of America

ABSTRACT

Opportunity, Challenges, and the Future demonstrates that personalized medicine can make patients healthier while lowering systemic costs. In addition to documenting a 62 percent increase since 2012 in the number of personalized medicines on the market, one report showed evidence in support of the field's most established benefits, which include:

1. Shifting the emphasis in medicine from reaction to prevention
2. Directing targeted therapy and reducing trial-and-error prescribing
3. Reducing adverse drug reactions
4. Revealing additional targeted uses for medicines and drug candidates
5. Increasing patient adherence to treatment
6. Reducing high-risk invasive testing procedures
7. Helping control the overall cost of health care

FDA carried that momentum into 2018 by clearing on March 6 an at-home personalized test marketed by 23andMe that provides information about a patient's personal risk of developing breast and ovarian cancer based on the presence or absence of mutations in the BRCA gene. In so doing, the agency bolstered the trend toward direct-to-consumer genetic testing, despite ongoing objections from some clinicians who believe that accurate interpretation of genetic test results requires a "learned intermediary."

The potential for personalized medicine is changing practice of medicine. It is no longer a discussion it's becoming reality in every branch of medicine. As this industry has seen an exponential growth and is poised for further growth because if advancements being made continuously.

Pharmaceutical and biotech companies have adopted and incorporated biomarkers growth into their corporate mission. The cost of diagnostic medicine for biomarkers has plummeted therefore making it feasible to test every individual who need personalized medicine information.

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PS-Lec- 0006

MOST CANCERS ARE CAUSED BY BAD LUCK; HOW WE MAY SCIENTIFICALLY PROVE THIS?

Prof Dr. Shahid Pervez
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Professor & Consultant, Section of Histopathology, Department of Pathology & Laboratory Medicine, Agha Khan University, Karachi Pakistan

ABSTRACT

There is no dearth of robust data correlating 'Environmental & Life style Factors' with various types of cancers. However, there are still a large number of cancers which arise out of blue with no known risk factors (1). These include some of the most lethal cancers like 'Brain gliomas', 'Pancreatic cancer', 'Ovarian cancers' and so many others. Question is then asked particularly by the patient; 'why am I suffering from this cancer in spite of healthy habits and no obvious risk factors? To explain this we need to go back to basics i.e., DNA. DNA is a fragile molecule that is continually under assault not only from agents in the environment & our diet but even from normal processes that takes place within the cell (2, 3). It is therefore critically important that damage to our genetic material is recognized and efficiently removed. It has been calculated that in a single day, DNA in one cell suffers about 20,000 single strand breaks and 10-20 double strand breaks. Single strand breaks are easy to repair as complimentary sequences are present, however double-strand breaks are difficult to repair as unlike both strands of the double-helix are affected, such that there is no immediately available template in the form of an intact complementary strand to use for correcting the lesion. It also involves the risk of losing a whole part of chromosome and potentially hundreds & thousands of genes (2, 3, 4). Most errors happen at the time of cell replication. Keeping in mind trillions of cells in human body, the propensity of DNA damage and DNA repair genes like BRCA1 & BRCA2 (4, 5) which also are prone to develop mutations, one actually ponders instead that 'Why cancer is so uncommon?'

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PS-Lec-0007

IMPROVING TUBERCULOSIS DIAGNOSIS: MULTIMODALITY INTEGRATIVE APPROACH

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ABSTRACT

Approximately, two billion people worldwide are infected with Mycobacterium tuberculosis (M. tb.), the etiologic agent of tuberculosis (TB). A tenth of the infected individuals develop active disease. Active pulmonary TB is an inflammatory disease and is increasingly viewed as an imbalance of host immune responses to M. tb. infection. The current frontline diagnostic methods including sputum smear (SS) microscopy, and X-ray, are insensitive, inefficient, cumbersome or too expensive. The most widely used test, SS microscopy (WHO standard), test has a low sensitivity. Therefore, there is an urgent need for low cost, efficient, high-throughput and accurate diagnostic approaches. We have developed immune biomarker-based TB diagnostic system in blood in pre-clinical nonhuman primate model and have extensively tested in TB patients. Data on immune biomarkers, microbiology, and CT imaging from proof-of-concept and subsequent field studies have shown that this approach will enable a scalable, flexible and cost-effective model for diagnostic applications. In addition, we have published that 10-plasma cytokine/chemokine biomarkers representing host immune-responses in TB patients, are not only gender biased but concentrations of some of these biomarkers (e.g., IP-10, MIG, IL-16, IFN-α and G-CSF) progressively decrease in patients which responded to anti-tuberculosis treatment (ATT) with a cocktail of several drugs (isoniazid, rifampin, ethambutol, and pyrazinamide or streptomycin - WHO Standard). These decreases strongly correlated with treatment success and can be used for monitoring efficacy of therapy - ATT is a drawn-out process (over many months), and early detection of patients who may not respond to therapy is important. Clinical studies in three developing countries (Pakistan, India and Uganda) have demonstrated that immune biomarker-based approach to TB is highly useful and can be successfully positioned as a blood test for diagnosis of all forms of TB (pulmonary, extra-pulmonary and pediatric) in comparison to the sputum based diagnostic tests that are limited for use in pulmonary TB. A validation trial in India, approved by the Indian Council for Medical Research (ICMR), New Delhi, is currently under way. One possible reason for not responding to ATT could be due to infection with multi-drug resistant (MDR) strain of M. tb. The standard culture-based drug sensitivity testing can take several weeks. Therefore, there is a need for rapid molecular tests. A test, based on multiplex gene amplification (multiplex PCR), of several M. tb. genes involved in drug resistance, and multiplex detection of the relevant gene mutations to detect resistance against four TB drugs will also be discussed.

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PS-Lec-0008

APPRAISAL AND REVALIDATION – A UK PERSPECTIVE

Dr. Muhammad Shafiq Gill
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ABSTRACT

The trust forms the basis of the relationship between doctor and patient. Historically it has been taken for granted that doctors remained competent and up to date unless fitness-to-practice proceedings decided otherwise. The medical profession in the UK has been wrestling with the concept of revalidation for decades. Enquiries into some high-profile medical scandals highlighted the need for robust system of medical revalidation. Medical Revalidation was launched in 2012 in the UK to strengthen the way doctors are regulated with the aim of improving quality of care provided to patients, improving patient safety and increasing public trust and confidence. It is based on a local evaluation of doctors' practice through appraisal and other supporting information. This talk reviews the process and outcomes of medical revalidation in the UK especially how it relates to pathologists.

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PS-Lec-0009

PATHOBIOLOGY OF PROSTATE CANCER AND TREATMENT OPTIONS

Prof Dr. Serajuddaula Syed
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ABSTRACT

We have indeed come a long way in our understanding of the pathology of the prostate over the past several decades and century. It goes without saying that in the past 20 years, there has been an exponential growth in our wealth of knowledge of the pathology of the prostate. Thanks to the hard work and dedication of several members of the ISUP. The current era of "omics" (genomics, metabolomics, pharmacogenomics, proteomics, ets.) and tissue-based biomarker research continues to evolve dramatically. The recent discovery of several key genetic alterations in prostate cancer also makes the potential molecular classification of this common tumor a distinct possibility in the not too distant future. In addition, recent advances in bioinformatics, microarray technology and nanotechnology will likely play a key role in the personalized management of patients with prostate cancer within the next 20 years.

PS-Lec-0010

MOLECULAR PATHOLOGY IN THE DEVELOPING COUNTRIES

Maj Gen (Retd) Suhaib Ahmed HI(M)
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ABSTRACT

Pathology has traditionally been a descriptive science. Pathologists like to give elaborate description of the gross and microscopic picture of a pathological lesion. With the availability of techniques to analyze proteins and more recently the nucleic acids we now know that at the submicroscopic level most pathological processes are caused by changes in the proteins or the genetic code. Most of these changes remain beyond the visible range of a microscope. Understanding of the molecular basis of disease provides a vast potential for diagnosis by using molecular analytical techniques. Consequently, molecular pathology is now recognized as a distinct entity.

The proteins as markers of disease are in use for the past several decades. The use of molecular genetic techniques in diagnostic pathology is a relatively new development. The past couple of decades are dominated by an ever-increasing use of PCR and its applications in the diagnosis of a wide range of diseases. PCR has made DNA analysis so simple that even small diagnostic labs can routinely offer sophisticated molecular genetic testing.

The major areas where molecular genetic techniques have made an impact include inherited, neoplastic and infectious disorders. In addition, it has also provided a strong basis for DNA profiling in forensic pathology and tissue typing for organ transplantation.

There is a general feeling that molecular genetic techniques are expensive, complex, and difficult to practice. Therefore, in a developing country like Pakistan the full potential of their use in diagnostic pathology has remained limited. Unfortunately, the development of newer and more complex techniques like droplet PCR, microarrays and NGS have further increased the gap between technology and its utility. In this challenging scenario the applications of molecular genetic techniques in a resource constrained setting need to focus on:

1. Identification of high priority areas.
2. Development of low-cost PCR applications.
3. Development of high-tech equipment at low cost.
4. Outsourcing of complex molecular genetic testing requiring expensive equipment.

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PS-Lec-0011

SIMPLIFY AND MAXIMIZE YOUR ROUTINE BY NEXT GENERATION SEQUENCING

Dr. Jeroen Marcel
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ABSTRACT

With the rapidly increasing amount of therapies available for treating Cancer patients there is an increased need to screen for multiple biomarkers. This additional information from a tumor allows for a well-educated decision on possibilities for treatment as well as personalized medicine. With technology advances it is now possible to screen for many different markers from a limited amount of tissue in a minimal amount time. Next generation sequencing (NGS) resolves the current day issues of iterative testing which is increasing cost and time and results in a lack of comprehensive profiling of a tumor. Here we describe some examples demonstrating why NGS is becoming the preferred choice for assessing tumors within a patient.

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PS-Lec-0012

EVOLVING WORLD OF ONCOLOGIC PATHOLOGY

Dr. Raphael Borok
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ABSTRACT

Massive parallel sequencing (NGS) has been revolutionizing our understanding of cancer biology, and is changing the approach to tumor diagnosis in pathology from a histologic to a genetic-based level, or more importantly to network-based level. Increasingly, pathology reports are incomplete without the addition of relevant genomic data.

Cancer is an acquired genetic disease, the result of an accumulation of molecular alterations in the genome of somatic cells, and our deeper understanding of tumor biology together with the ongoing development of treatments targeting molecular alterations has enabled the emergence of personalized medicine in oncology. Tumor genotyping is helping oncologists to individualize

treatments, and tumor specific DNA alterations provide sensitive biomarkers for disease detection and monitoring. Analytical tools, including machine learning/ AI, are helping to identify patterns in tumor genomic landscapes that are defining new tumor subtypes. Sequencing today routinely includes DNA sequencing, RNA sequencing (whole transcriptome, including protein coding and non-coding RNA) an assessment of tumor genetic instability (microsatellite instability and total mutational burden) and IHC for PD-L1 expression, and sequencing of corresponding normal patient DNA. Information technology and analytical tools are also enabling a transition from a dependence for progress on traditional randomized clinical trials alone, to large community-based ongoing stage 4 clinical trials. A patient can now be compared online to cohorts of patients with similar tumors and tumor genomic landscapes to see the various regimens that were used to treat them, and to assess how they responded to the selected regimens.

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PS-Lec-0013

QUALITY MANAGEMENT AND ACCREDITATION IN CLINICAL LABORATORY

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ABSTRACT

The maintenance of a quality management system is crucial to a laboratory for providing the correct test results every time. Important elements of a quality management system include: Documentation Standard Operating Procedures (SOP's), Quality Control samples and External Quality Assessment Scheme. Concept of total quality management (TQM) is closely interlinked with good laboratory practices and goes far beyond the widely practiced conventional Quality Control (QC) procedures. TQM includes Technical accuracy and precision, equipment and supplies, staff training and skill, financial management (cost effectiveness), lab safety, communication etc. Quality improvement in the modern clinical laboratory environment entails the continuous inspection and refinement of processes to ensure the efficient delivery of services that meet the needs and expectations of those who use them. College of American (CAP) Laboratory Accreditation Program is widely recognized as the 'gold standard' and has served as a model for various federal, state, and private laboratory accreditation programs throughout the world. Its inspection program is internationally recognized and the only one of its kind that utilizes teams of practicing laboratory professionals as inspectors. Designed to go well beyond regulatory compliance, the program helps laboratories achieve the highest standards

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HISTOPATHOLOGY SCIENTIFIC SESSION ABSTRACTS

SS-Histo-0001

PERSONALIZED MEDICINE IN BREAST CANCER

Dr Shahid Bashir
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ABSTRACT

Personalised breast cancer management is treatment tailored to the individual and starts with screening for the disease. Treatment has always been adapted to the patient what is changing is that it is increasingly being tailored to the molecular features of tumours. From BRCA and oncotype Dx an increasing number of molecular markers are being investigated which will determine the chemotherapy/radiotherapy given. The pathologist needs to not only own this and provide the source of expertise and interpretation but also educate other physicians.

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SS-Histo-0002

RECENT ADVANCES IN REPORTING OF SALIVARY GLAND CYTOLOGY – THE REAL BASIC FACTS & LIMITATIONS

Prof Mulazim Hussain Bukhari
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ABSTRACT

No doubt, there are lots of limitations of FNAC of any lump, especially salivary gland, due to its diversity of cytological architecture, but the use of FNAC is helpful in the early diagnosis of lesions. The use of this modality is rapid and safe. With limited complications by the use of FNAC, the preoperative distinction of benign and malignant lesions is easy. The use of FNAC is usually specific and sensitive in the diagnosis of salivary glands lesions. It has high diagnostic accuracy for benign lesions and lower for malignant tumors. Nowadays, the material of FNAC can replace the need for frozen sections, where ever the facility is not available. It is useful to aid in the conservative management of benign or low-grade malignancies. It allows to carry out palliative treatment for high grade tumors and metastases. In spite of these advantages, there is no uniform and widely accepted reporting system.

Establishing a common reporting system (Milan) will promote the education and research in salivary gland cytology just as any other diagnostic cytology systems have done in their respective areas of cytopathology. Creation of such reporting systems is a natural consequence of the maturation of evidence-based literature and clinical experience.

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SS-Histo-0003

DELIVERING FROZEN SECTION SUPPORT TO MULTIPLE HOSPITALS - CHALLENGES AND OPPORTUNITIES

Dr Omer Chughtai,
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ABSTRACT

Frozen section is an essential component of pathology support for surgery. Ideally the frozen section lab and pathologist should be available within the hospital where the surgery is taking place. In settings where in house frozen section facility is not available, alternative arrangements have to be made to make the facility available for patient care.

In this presentation we will share the experience of a centrally located pathology lab in delivering frozen section support to multiple hospitals in Lahore. The talk will focus on challenges faced in establishing and running such a facility, and the positive impact on patient care that is still possible in this setting.

SS-Histo-0004

AN UPDATE ON ENDOCRINE PATHOLOGY - ADRENAL AND PARATHYROID TUMOURS: DIAGNOSTIC CHALLENGES AND RECENT DEVELOPMENTS

Dr Mariam Khan
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ABSTRACT

This talk will focus on the recent developments and diagnostic challenges of Adrenal and Parathyroid gland tumours in the light of the World Health Organization (WHO) 2017 classification of endocrine tumours. It will discuss the criteria for predicting behavior in phaechromocytoma and paraganglioma. These tumours may all have metastatic potential and are therefore risk stratified. Different systems are used to predict their behavior and include PASS and GAPP systems. These tumours are also increasingly recognized as hereditary with

genotype/phenotype correlations. Adrenal cortical carcinoma has no well-defined histological features to confirm malignancy. Various systems are available that include Weiss, modified Weiss and a 2-step reticulin method. Various predictors for prognosis have been suggested including mitoses, Ki67 and the Weiss and Helsinki scores etc. Adrenocortical tumours with unusual features, such as oncocytic, myxoid etc can be challenging. Different criteria have been suggested to predict behaviour in oncocytic and paediatric adrenocortical tumours. Parathyroid carcinoma is a very rare tumour and its diagnosis can be very challenging as the histological features can be subtle and often mimicked by degenerative changes especially related to pre-surgery procedures. This talk aims to outline a practical approach as to how to report them in the most clinically useful way with some practical examples.

SS-Histo-0005

EWING LIKE SARCOMAS

Brig Hafeez Uddin
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Consultant Histopathologist & Associate Prof of Pathology
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ABSTRACT

Ewing-like sarcomas (ELS) are a heterogenous group of tumors that affect pediatric and young adult patients. These tumors share a significant morphologic, Immunohisto-chemical and clinical overlap with ESFT, thus the differential diagnosis is challenging. The Ewing Sarcoma Family of tumors are almost always characterized by reciprocal translocations between *EWSR1* and a gene of the *ETS* family of transcription factors. However, these tumors lack these molecular alterations. A subset of ELS harboring the *CIC-DUX4* or *BCORCCNB3* fusions has been described recently. The subset with CIC-DUX4 gene fusion is found to harbor rearrangement in CIC and DUX4.These tumors have a more aggressive clinical course and are less responsive to traditional Ewing chemotherapy. While the Ewing like sarcoma with BCOR - CCNB3 gene fusion, result of a cytogenetically cryptic chromosomal X inversion translocation have some features of Ewing sarcoma but also display atypical features, including spindle cells. CCNB3 immunohistochemistry is found to be positive in this subgroup of sarcoma. Behavior in these tumors is not as established given their rarity. However, there are some reports which show better response to the traditional chemotherapy.

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SS-Histo-0006

STRATIFIED MEDICINE IN CANCER: THE ROLE FOR HISTOPATHOLOGIST

Brig Tariq Sarfraz
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ABSTRACT

Histopathologists (are also called Cellular Pathologists) have been supporting and refining the process of accurate disease diagnosis and its classification for decades. The methods such as tinctorial stains, immunohistochemistry and in situ hybridization are adopted in order to supplement the morphological assessment of tumours as part of diagnostic process. Stratified cancer medicine involves predictive analysis; the characterization of tumours according to the presence or absence of specific molecular abnormalities that are associated with different treatment response, in order to offer appropriately targeted therapy and avoid unnecessary treatment.

The recent advances in genomic technology have opened new possibilities for molecular taxonomy in cancer. This is a rapidly evolving area, which is having a direct impact on histopathology practice. This represents an important part on the wider concept of delivering more personalized or precision medicine across many different disease areas in the current postgenomic era, since the elucidation and publication of the first human genome over a decade ago. The aim of this talk is to summarize the advances in this area with focus on solid tumours in adult patients. Sample requirements, technologies adopted, progress toward routine delivery of predictive molecular analysis, its application in clinical practice and challenges faced will be highlighted.

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SS-Histo-0007

PROBLEMS IN DIAGNOSING CD20 NEGATIVE B-CELL LYMPHOMAS

Dr Arsalan Ahmed,
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ABSTRACT

Immunohistochemistry plays an integral part in the diagnosis and subclassification of hematolymphoid neoplasms. CD20 and CD3 are the most commonly used immunohistochemical markers for identification of B cells and T cells, respectively, and they are believed to always yield specific staining. However, problem arises when these reliable markers exhibit unusual expression which

can lead to misdiagnosis. This talk focuses on diagnostic pitfalls related to the use of immunohistochemistry for CD20 in hematolymphoid neoplasms, and specifically on diagnostic challenges that arise when (1) CD20 is not expressed in B-cell lymphomas and (2) how to still get to the right diagnosis

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SS-Histo-0008

EVOLUTION OF LYMPHOMA CLASSIFICATION

Brig Shahid Jamal (Retd)
Professor & Head of Department of Pathology Watim Medical & Dental College, Rawalpindi Pakistan

ABSTRACT

The lymphomas comprise a heterogeneous group of cancers with diverse aetiologies, treatment pathways and outcomes. Right after it was described by Thomas Hodgkin as disease entity, the knowledge of disease progressed. The biological understanding about the relationship between these complex malignancies, the bone marrow, the immune system and the cellular and genetic basis of malignant transformation increased and was incorporated in different classifications. The lymphoma classifications are unique that no other disease has been classified and reclassified, so many times during its evolution, as the lymphoma was. How its understanding progressed in relation to its classifications will be presented.

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SS-Histo-0009

AN UPDATE ON THYROID TUMOURS

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ABSTRACT

Thyroid neoplasia is a challenge for all pathologists. The latest 2017 WHO classification is an attempt to provide a unified and objective approach to the different problematic lesions. Non-invasive follicular tumour with papillary like nuclear features or NIFTP as it is abbreviated is the new name given to one class of borderline lesions, the caveat being that strict criteria are required to be adhered to. The use of the term “encapsulated follicular variant of papillary carcinoma” without further qualification is discouraged. There is now a paradigm shift with much more emphasis on vascular invasion as compared to nuclear features in the diagnosis of follicular patterned lesions. Hurthle cell lesions have gained their own place in the scheme of tumours rather than being a type of follicular neoplasm. Poorly differentiated carcinoma is diagnosed using the Turin consensus criteria with an algorithmic approach. The importance of necrosis and mitoses have been highlighted. A brief overview of the above will be presented

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SS-Histo-0010

PERSONALIZED LUNG CANCER DIAGNOSIS – PRACTICAL CONSIDERATIONS

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ABSTRACT

Lung cancer is the second most frequent type of cancer but by far the most frequent cause of cancer-related deaths. A large number of non-small cell lung carcinoma (NSCLC) cases have known oncogenic driver mutations or translocations some of which can be exploited for targeted therapies. The era of targeted cancer therapeutics has brought forth new challenges for pathologists and pathology laboratories. Pathologists are increasing finding themselves at the center of diagnostic work up for NSCLC, with an expectation to ensure judicious use of small amount of diagnostic tissue and an ability to coordinate prognostic and predictive information from a multitude of diagnostic techniques. Next generation sequencing (NGS) is a powerful tool for molecular characterization of solid tumours however its relationship with pre-existing diagnostic modalities needs to be further understood. The ever-expanding field of proteomics and genomics demands a review of current pathology training systems so that future pathologists are better equipped to deliver an integrated cancer diagnostics service. This talk explores practical challenges faced by pathologists in ensuring an intergraded diagnostic approach to NSCLC.

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SS-Histo-0011

LIVER PATHOLOGY UPDATE

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HISTOPATHOLOGY FREE PAPER ABSTRACTS

FP-Histo-0001

CONCORDANCE OF CYTOMRPHOLOGICAL FEATURES OF CERVICAL GRANULOMATOUS LYMPHADENITIS ON FINE NEEDLE ASPIRATION BIOPSY WITH GENEXPERT FOR MYCOBACTERIUM TUBERCULOSIS ON ASPIRATED MATERIAL

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ABSTRACT

Objective: The objective of the study is to determine concordance of different cytomorphological features of cervical granulomatous lymphadenitis on fine needle aspiration biopsy with GeneXpert for mycobacterium tuberculosis (MTB).
Study Design: Cross sectional correlational study.
Place and duration of the study: Department of Histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from November 2016 through November 2017.
Material and Methods: Fine needle aspirates of a total 51x patients with cervical granulomatous lymphadenitis
were included in the study and the cytology was compared with geneXpert, keeping geneXpert as gold standard. After obtaining FNA aspirates from cervical lymph nodes, the specimen was evaluated for various patterns of granulomatous inflammation. The aspirate was sent to the microbiology department for confirmation of presence of MTB by PCR (geneXpert), results of which were then compared.
Results: Out of 51 samples evaluated for cervical granulomatous lymphadenitis, 38 (74%) samples were positive for MTB, while 13 (26%) samples were negative by GeneXpert. 15 samples showed granuloma formation on FNA. 21 samples showed granulomatous caseous necrosis on FNA. 15 samples showed suppurative granulomatous lymphadenitis on FNA. Data was analyzed using SPSS version 24. Mean age was 30±2 SE years and the range was 4 to 72 years. Granulomatous inflammation showed only 40% positivity (6/15), Chronic caseating granulomatous inflammation showed 95% positivity while granuloma with suppuration showed 80% positivity for MTB on PCR (12/15) (P value 0.001)
Conclusion: FNA is a rapid, safe, inexpensive, easily available and an outpatient procedure for the diagnosis of MTB in cases of cervical lymphadenitis. Significant association of caseous granulomatous and suppurative granulomatous lymphadenitis with MTB is seen & the results can be safely implemented for initiation of ATT in country like ours where advanced diagnostic facilities are not easily accessible for a common man. Granulomatous inflammation however should be further evaluated to rule out the underlying cause.
Key Words: Fine Needle Aspiration Cytology, Cervical Lymphadenitis, PCR, Granulomatous inflammation. Caseous necrosis, Suppurative lymphadenitis.

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FP-Histo-0002

PATHOLOGICAL SPECTRUM OF SOLITARY FIBROUS TUMOR: A STUDY OF 25 CASES DIAGNOSED AT SHIFA INTERNATIONAL HOSPITAL, ISLAMABAD

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ABSTRACT

Objective: To determine the clinicopathological and immunohistochemical characteristics of solitary fibrous tumors.
Study design: Retrospective, Descriptive.
Material and Methods: Hematoxylin and eosin and immunohistochemical slides of solitary fibrous tumor during 2012 to 2018 were retrieved from the record. Clinical information including age, gender, clinical symptoms and tumor location was collected. Histopathological features like hypercellularity, increased mitosis, cytological atypia and infiltrative margins were analysed. Diagnosis was based on morphology and immunohistochemistry.
Results & conclusion: Among the 25 patients 12 were male and 13 were female. Of the specimen received, 10 were excisional biopsies, 8 were core biopsies and 7 were resection specimens. 15 cases were benign and 10 were malignant. Among the benign cases 10 were female and 5 were male and in malignant cases 7 were male and 3 were female. Most common site for benign tumors was thoracic cavity (8 cases), 4 tumors were in the abdominal region, one from nape of neck, one from the thigh and one from perioccipital parasagittal region. Among malignant cases 3 were from abdominal cavity, 3 from thoracic region, 2 from spinal region, 1 from pelvic and one from retrobulbar region.
Key Words: Solitary fibrous tumor, tumor necrosis, infiltrative margins

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FP-Histo-0003

IMMUNOHISTOCHEMICAL EXPRESSION OF ANDROGEN RECEPTOR IN TRIPLE NEGATIVE BREAST CARCINOMA AT AFIP, RAWALPINDI

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ABSTRACT

Objective: To determine the frequency of immune-histochemical expression of androgen receptor in triple negative breast carcinoma.

Study Design: Descriptive, Cross sectional study.

Materials & Methods: This study included 30 cases confirmed as triple negative breast carcinoma at Armed Forces Institute of Pathology, Rawalpindi from June 2017 to December 2017. Anti-androgen receptor antibody was applied and assessed. Positive expression was defined as greater and equal to 10% nuclear immunostaining. SPSS 21 was used for analyzing data. Descriptive statistics and frequencies were calculated.

Results: Total 30 cases of TNBC were included and all patients were female. Age of patients ranged between 21 to 72 years with a mean age of 46.35 years and a standard deviation of ± 13.4. Androgen receptor expression was positive in 8 cases (27%) of TNBCs. Out of these AR positive TNBCs; most of the cases were of histological subtype invasive ductal (mammary) carcinoma, NST and 4 cases (50%) were of histological grade 3.

Conclusion: Androgen receptor expression is observed in a significant number of triple negative breast carcinoma cases. Such patients can be selected as candidates for anti-AR targeted therapy.

Keywords: Androgen receptor, Triple negative breast carcinoma.

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FP-Histo-0004

STROMAL CD10 IN INVASIVE BREAST CARCINOMA; CORRELATION WITH ER, PR AND HER 2 NEU STATUS

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ABSTRACT

Objectives: To access the correlation of ER, PR and HER2-neu with stromal CD10 in Invasive Breast Carcinomas.

Study design: Descriptive/ cross-sectional.

Place and duration of study: Two-years, Pathology Department, Federal Government Polyclinic, Islamabad / Pakistan Institute of Medical Sciences-PIMS (SZABMU) Islamabad.

Material and Methods: A total of one hundred and seventy-one (n=171) female patients undergoing surgical intervention for invasive carcinomas of breast were included. 5µm thick slices were used for immunohisto-chemical staining for determining ER, PR and stromal CD-10. ER and PR expression status was further analyzed using Allred scoring system in each case by taking into consideration the proportion of positive cells and staining intensity. The correlation between stromal CD-10 and ER/PR status was measured. Data was analyzed using SPSS version 17. Chi-square test was employed to assess the significant of association between CD-10 and ER/PR expression and tumor grades. A P-value of ≤ 0.05 was considered significant. The Pearson correlation coefficient was calculated to assess the strength of correlation between C-10 and other parameters.

Results: Mean age of study participants was 48.1 years ± 10.4 SD. All the specimens were diagnosed as invasive ductal carcinoma except a single case of invasive lobular carcinoma. There were 8.8% (n=15/171) specimen diagnosed with grade I, 71.3% (n=122/171) grade II and 19.9% (34/171) with grade III. Our results showed that 77.8% (n=133/171) cases were CD-10 positive, (31% n=53/171 were weak positive and 46.8% n=80/171 were strongly positive). A total of 66.1% (n=113/171) cases were ER, 6.7% (n=97/171) cases were PR and 29.2% (n=50/171) were Her-2-Neu positive. A significant association between ER-negative status and CD-10 intensity was observed (65.5%, n=38/58 of ER negative cases expressed strongly positive CD-10 in the stroma vs 37.2%, n=42/113 ER positive cases; P=0.001). An association, although statistically not significant, was also observed between higher tumor grades and CD-10 intensity (55.9% n=19/34 of grade III, 45.1% n=55/122 of grade II expressed strongly positive CD-10 in the stroma compared with 40.0% n=06/15 grade I cases; P=0.258). The association of CD-10 with PR and HER-2-NEU was not significant (P=0.841 and P=0.749 respectively). Stromal CD-10 expression was found to be negatively correlated with ER-expression, which was fairly strong and significant (R= -0.304 at P=0.001). Stromal CD-10 expression was found to be positively correlated with tumor grades, which was also significant (R=0.159 at P=0.038). Stromal CD-10 expression was correlated positively with PR and Her-2-neu status but it was weak and non-significant (R=0.065 at P=0.396 and R=0.082 at P=0.286, respectively).

Conclusions: Present study results showed that stromal CD10 expression in invasive carcinoma of the breast is significantly associated with ER negativity and higher tumor grade. It may be potential prognostic marker and a target for development of new therapies.

FP-Histo-0005

ABSTRACT: EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) OVEREXPRESSION IN TRIPLE-NEGATIVE BREAST CANCER

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ABSTRACT

Objective: Triple-negative breast cancers are a poor prognostic group of breast cancers that don't respond to conventional hormonal and her2neu targeted therapy. A subset of triple-negative breast cancer is known to overexpress epidermal growth factor receptor (EGFR); however prognostic significance of this biomarker has not been widely studied in our population. Therefore, we aimed to evaluate the frequency of EGFR overexpression in triple-negative breast cancer in our setup and its association with prognostic and predictive factors.

Study Design: Cross sectional study

Material & Methods: We performed EGFR immunohisto-chemistry on 150 cases of triple-negative breast cancers. Intensity and percentage of EGFR expression were combined to formulate an EGFR score, that was compared with prognostic features of breast cancer and recurrence status of patients.

Results: Positive EGFR expression was noted in 18.7% (28 cases); out of which 16% (24 cases) showed low EGFR expression, whereas high EGFR expression was seen in 2.7% (4 cases). No significant association of EGFR expression was noted when compared with various clinicopathological parameters and recurrence status of the patients.

Conclusion: We found EGFR protein expression in 18.7% of cases while high expression was seen in only 2.7 % cases of triple-negative breast cancer which may harbor underlying genetic alterations like altered EGFR gene copy number, chromosome 7 copy number or average EGFR gene: chromosome 7 ratio; therefore, we suggest that molecular tests like FISH to evaluate EGFR molecular alterations. It should be performed in EGFR over expressing triple negative breast cancers in our setup to identify patients that can benefit from anti-EGFR targeted therapy. Moreover, regional difference in EGFR expression (high expression in Chinese population compared to our population) may be due to different underlying genetic alterations in triple-negative breast cancers, further necessitating a need for devising personalized therapeutic protocols for locoregional population.

Key Words: Triple-negative breast cancers, EGFR, CK5/6, epidermal growth factor.

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FP-Histo-0006

C1Q NEPHROPATHY: A DISEASE WITH DIVERSE HISTOPATHOLOGICAL PRESENTATIONS: STUDY AT A TERTIARY CARE CENTRE"

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ABSTRACT

Introduction: C1q nephropathy is a rare glomerulopathy, usually seen in children and young adults. Worldwide it has a very low prevalence; varying from 0.2 to 2.5%. The etiopathogenesis of this rare disease is unclear. It has different clinical presentations and may show varying morphological appearances. It is defined as the presence of mesangial immune deposits that stain dominantly or co-dominantly for C1q accompanied by negative antinuclear antibodies in patient's serum and absence of clinical evidence for SLE. Even though, more than three decades have passed since this entity was first explained, still it remains a dilemma for many due to rarity of this entity.

Material and Methods: We carried out a retrospective study from 1st January 2011 to 30th December 2015. All cases diagnosed as C1q nephropathy were retrieved from hospital's computerized data base. Their clinical profiles, morphology and immunohistochemical profiles were studied.

Results: Over this period a total of 21 cases were diagnosed as C1q nephropathy. Mean age of the patients was 33.8 years. The most common clinical presentation was nephrotic syndrome seen in 15(71.5%) patients. The most frequent morphological pattern seen was minimal change disease (MCD) in 8(38.1%) cases. All cases showed dominant 12(57.1%) or codominant 9 (42.9%) mesangial C1q deposition.

Conclusion: C1q nephropathy is a rare but distinct clinicopathological entity with varying presentations and histological patterns. It is primarily diagnosed on the basis of immunofluorescence findings with a dominant or codominant fluorescent intensity for C1q. It is recommended that C1q nephropathy be sought preferably with immunoflourescence staining of biopsies for immune reactants. Studies from this part of the world are strongly recommended to predict clinical outcome and treatment options.

Key Words: C1q nephropathy, immunoflourescence, immune reactants, histopathological patterns.

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FP-Histo-0007

ANALYSIS OF INTEROBSERVER VARIABILITY IN CORE BREAST BIOPSIES OF CATEGORY B3 AND B4 AT A TERTIARY CARE CENTRE

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ABSTRACT

Objectives: To evaluate interobserver variability in core breast biopsies of category B3 and B4 at a tertiary care centre

Study Design: Retrospective, observational, cross-sectional study.

Place and Duration of Study: Histopathology Department, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from March 2017 to June 2018

Materials and Methods: A total of thirty cases of histologically confirmed lesions of category B3 and B4 on core breast biopsies from March 2017 to June 2018 were retrieved from archive of Histopathology department, AFIP Rawalpindi. Patients' age, histologic diagnosis and reporting B category were noted. The selected cases were anonymized and distributed among 3 pathologists for independent review. Participants were blinded to the interpretations of other study pathologists and consensus-derived reference diagnosis. The cases were reviewed by the participating pathologists. The results were analyzed and overall concordance rate, discordance, overinterpretation rate and under interpretation rate were calculated.

Results: A total of 30 (n=30) patients were enrolled of which 22 were assigned category B3 (73%) and 8 were assigned category B4 (27%) on initial microscopic evaluation (consensus-derived reference diagnosis). The age range of the study patients was 25 to 85 years. The overall concordance rate of diagnostic interpretations of participating pathologists compared to the reference diagnosis was 63% (19/30). Among these the average concordance rate compared to reference diagnosis among category B3 lesions was 73% (16/22) and among category B4 lesions was 37.5% (3/8). The overall rate of disagreement of diagnostic interpretations of participating pathologists compared with the reference diagnosis was 37% (11/30). Among these the average rate of disagreement/discrepancy compared to reference diagnosis among category B3 lesions 27% (6/22) and among category B4 lesions was 62.5% (5/8). The overall overinterpretation rate was 44% and overall under interpretation rate was 56%. The overall rate of unanimous agreement of independent diagnoses among the three panel consultants was 50% (15/30). Among these 15 cases 12 were assigned category B3 and 3 were assigned category B4. The overall rate of disagreement of independent diagnoses among the three panel consultants was 50% (15/30).

Conclusion: In this study of pathologists, in which diagnostic interpretation was based on a single breast biopsy slide, overall agreement between the individual pathologists' interpretations and the expert consensus–derived reference diagnoses was 63% and disagreement was 37%, with higher level of concordance for category B3 and lower levels of concordance for category B4 lesions. Further research is needed to understand the relationship of these findings with patient management.

Key Words: Interobserver variability, core breast biopsy, category B3 and B4.

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FP-Histo-0008

PROGNOSTIC SIGNIFICANCE OF P16 IMMUNOHISTOCHEMICAL EXPRESSION IN UROTHELIAL CARCINOMA

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ABSTRACT

Objective: p16 is the protein product of most commonly involved gene in bladder carcinogenesis. Therefore, we performed an immunohistochemical study to evaluate association of p16 overexpression with prognostic parameters in bladder cancer.

Study design: Cross sectional study.

Materials and Methods: p16 immunohistochemistry was performed on 121 cases of bladder cancer and association with tumor grade, lamina propria invasion, muscularis propria invasion and survival status was noted.

Results: Low expression of p16 was noted in 86% (104 cases), whereas 14% (17 cases) revealed high p16 expression. We found significant association of p16 expression with tumor grade (p=0.000), muscularis propria invasion (p=0.001), lamina propria invasion (p=0.001) and survival status (p=0.020). Univariate binary logistics showed that low grade tumors were less likely to express high p16 expression as compared to high grade tumors. Similarly, patients with lamina propria and muscularis propria invasion were more likely to exhibit high p16 expression. Significant association of high p16 expression was noted with worse long-term survival (p=0.020), while univariate logistic regression showed that patients with low p16 expression were at low risk (HR=0.194) to die of disease as compared to patients with high p16 expression.

Conclusion: p16 is an important biomarker in bladder cancer as it can be used for prognostic stratification of patients with bladder cancer. Moreover, we suggest that molecular studies should be performed in our population in order to correlate abnormal p16 expression with underlying gene mutations.

Key Words: Bladder cancer, p16, Urothelial carcinoma, Muscularis propria invasion,

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FP-Histo-0009

EVALUATION OF ARGINASE-1 AS AN IMMUNOHISTOCHEMICAL MARKER FOR HEPATOCELLULAR DIFFERENTIATION- A STUDY OF 57 CASES

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ABSTRACT

Objective: To study the immunohistochemical expression of Arginase-1 in suspected primary and metastatic hepatocellular carcinoma.

Study design: Retrospective descriptive study.

Material and Methods: The study was performed in histopathology department at Shifa International Hospital, Islamabad. All cases from 1st January 2017 to 30th June 2018 in which immunohistochemical stain Arginase-1 was applied were included in the study. This stain was applied in cases in which primary or metastatic HCC was suspected.

Results: A total of 57 cases were included in the study. 34 cases showed Arginase-1 positivity and 23 were negative. Out of these, 22 turned out to be primary HCC, 07 were metastatic HCC and 28 other tumors. Among the primary HCC cases all were Arginase-1 positive, 75% were Hep Par-1 positive and 71.4% showed Glypican-3 positivity. All metastatic HCC were positive for Arginase-1 and Hep Par-1. Two out of other 28 cases showed aberrant positivity for Arginase-1. One was high grade serous carcinoma of ovary and other was well-differentiated neuroendocrine tumor. Two cases of hepatoblastoma from other tumors group showed positivity for Arginase-1 and Hep Par-1.

Conclusion: Arginase-1 is robust marker for hepatocyte differentiation but it can show occasional positivity in other tumors.

Key Words: Arginase-1, Hepatocellular carcinoma, Hep Par -1

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FP-Histo -0010

CHARACTERIZING KIDNEY STRUCTURES IN HEALTH AND DISEASES USING EOSIN FLUORESCENCE FROM HEMATOXYLIN AND EOSIN STAINED SECTIONS

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ABSTRACT

Objective: In this study, the use of eosin fluorescence to visualize and quantify histopathological changes was elucidated in a glycerol-induced model of acute kidney injury (AKI) in mice.

Study Design: It was and animal experimental study.

Material & Methods: AKI was induced in BALB/c mice by intramuscularly injecting 10 mL/kg of 50% glycerol after 24 h of water deprivation and, after another 24 h, the kidneys were dissected out. After H&E staining, kidney tissue sections were examined under a fluorescent microscope using a double channel filter cube (FITC and TxR).

Results: The H&E fluorescence pattern of normal kidney structures was characterized as the fluorescence patterns varied between different parts of the kidney and could be used for the structural identification of different parts of the kidney. Interestingly, in the injured kidney sections, a striking increase in the red fluorescence was noticed only in the damaged areas. In the damaged kidney sections, casts deposited inside tubules showed bright yellow fluorescence, whereas fibers showed strong green fluorescence. In vitro experiments were also performed in order to understand possible mechanisms underlying this phenomenon.

Conclusion: It was concluded that eosin fluorescence can be used to quantify the degree of damage to kidney tissue which, in turn, may help in assessing the kidney-protective effects of unknown compounds and plant extracts.

Key Words: Acute kidney injury, eosin fluorescence, glycerol-induced acute kidney injury, haematoxylin and eosin staining.

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FP-Histo-0011

ASSESSMENT OF MICROSATELLITE INSTABILITY IN ENDOMETRIAL CARCINOMA BY IMMUNOHISTOCHEMICAL EXPRESSION OF MLH1, PMS2, MSH2 AND MSH6

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ABSTRACT

Objective: To determine frequency of Microsatellite instability by expression of MLH1, PMS2 MSH2 and MSH6 in endometrial carcinoma.

Study design: Descriptive, cross-sectional

Place and duration of study: Armed Forces Institute of Pathology (AFIP), from 1st September 2017 to 28th February 2018.

Material and Methods: Thirty-two cases of endometrial cancer diagnosed on immunohistochemistry were included in the study. Patient's age, tumor type and FIGO grade were noted. Microsatellite instability was assessed by observing the loss of expression of MLH1, PMS2, MSH2 and MSH6 by immunohistochemistry (IHC). The data was analyzed by using computer software program SPSS version 24. Descriptive statistics and frequencies were calculated.

Results: The age of patients ranged between 30 to 80 years with a mean of 58.9 and standard deviation of ±10 years. The predominant histological type of cancer was endometrioid adenocarcinoma, NOS in majority of the

cases (n = 31) with one case of adenocarcinoma with squamous differentiation. Most common FIGO grade was Grade I (n = 27), followed by Grade II (n=4) and Grade III (n=1). Out of 32 cases, 28 (87.5%) cases showed abnormal expression of any of mismatch repair proteins while 4 (12.5%) cases retained expression of all proteins. The loss of expression rate of MLH1 protein was 31.25% (10/32), PMS2 protein was 18.75% (6/32), MSH2 protein was 68.75% (22/32) and MSH6 protein was 0% (0/32).

Conclusion: A significant percentage of cases showed loss of expression of MMR proteins by immunohisto-chemistry. These patients can be selected for further molecular testing for confirming the diagnosis of Lynch syndrome, subsequent genetic counseling and prevention of Lynch syndrome related cancers.

Key Words: Endometrial carcinoma, Immunohisto-chemistry, Microsatellite instability, Mismatch repair proteins.

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FP-Histo-0012

GENETIC AND BIOCHEMICAL PROFILING OF PALB2 WITHIN PALB2-BRCA2 INTERFACE IN PAKISTANI BREAST CANCER PATIENTS

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ABSTRACT

Objective: To determine the diagnostic accuracy of Cannabinoids testing by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS) in human hair and to compare it with urine for detection of cannabis use in civil heavy vehicle drivers

Design: Diagnostic accuracy (validation) study

Place and duration of study: This study was carried out at Department of Forensic Medical Sciences Laboratory (FMSL), Armed Forces Institute of Pathology Rawalpindi, Pakistan from February to November 2017

Materials and Methods: Hair and urine samples of 151 civil heavy vehicle drivers were collected from various areas of Punjab. Sampling technique was non-probability convenient. About 10 ml of urine was collected from each subject and stored at -20 °C. Hair strands were and collected kept at room temperature. Separation of compounds was done on Agilent Poroshell and analyzed on a 6460 Triple Quadrapole LC-MS along with software Mass hunter ©.

Results: All the 151-male civil heavy vehicle drivers, who were included in the study, were categorized into three main groups. There were 69(45.7%) truck drivers,43(28.5 %) twenty-wheeler drivers and 39(25.8%) bus drivers. Mean age was 36±10.82 years. Paired t-test was applied to check mean difference between the two tests' concentration (i.e urine and hair analysis for cannabis) which shows significant difference at **p<0.001**. Various parameters of diagnostic accuracy in hair and urine samples were: Sensitivity (96% and 62%), Specificity (93% and 95%) Positive Predictive Value (88% and 87%), Negative Predictive Value (97% and 82%) respectively. Overall diagnostic accuracy of Cannabinoids in hair was 94% while in urine it was 83%. Receiving Operating Characteristics (ROC) curve was plotted which showed area under curve of 0.96 and 0.79 for hair and urine respectively.

Conclusion: This study highlights the importance of hair as an alternative biological matrix due to its good diagnostic yield, non -invasive specimen collection and analyte stability, as well as wider detection period compared to urine.

Key Words: Cannabinoids testing in hair, liquid chromatography- tandem mass spectrometry (LC-MS/MS), diagnostic accuracy.

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FP-Histo-0013

CORRELATION OF ER, PGR AND HER-2 STATUS WITH PROLIFERATION INDEX KI-67 IN BREAST CANCER. THE MOLECULAR ICEBERG

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ABSTRACT

Objective: Hallmark of every cancer is unrestrained proliferation. Ki-67 is a biomarker that reflects cell proliferation. The heterogeneity of Breast cancer has been highlighted with the advent of molecular studies. As with all grading systems, mitotic activity is an indicator of aggressiveness of tumor. Ki-67 is an emerging marker that can be interpreted by immunohistochemistry and highlights the cells undergoing mitosis. ER, PgR and Her-2 have significant prognostic and predictive value as they are implicated in molecular grouping and subsequent therapeutic decision making. Incorporation of Ki-67 with these markers can further potentiate their clinical utility.

Material and Methods: The study was conducted in the Pakistan Institute of Medical Sciences (PIMS/FGPC) - Islamabad affiliated with Shaheed Zulfiqar Ali Bhutto Medical University, in the Department of Histopathology from April 2016 till March 2018. Total 178 cases of breast carcinoma were included in the study the tissue was processed and stained with Hematoxylin and Eosin (H&E), subsequently followed by histopathological interpretation. Immunohistochemistry was performed to assess expression of Ki-67. Immunoreactivity of Ki-67 was recorded as low, if less than or equal to 14 %, intermediate for 15% to 30%, high for more than 30%

Results: Total 178 breast cancer cases were collected during 24 months of study period. All patients were females. Initial histologic grading was done on the basis of microscopic findings. Most of the patients 100/178 (56.18

%) fall in age group of 41 to 60 years Mean Ki-67 index was 44.56 % (Range 5 % - 95 %). SD value for proliferation index was 24.87. ER, PgR immunoexpression was seen in 104/178(58.43%) cases and 83/178 (46.63%) cases, respectively. Her-2 positivity was observed in 58/178 (32.58%) cases. Invasive ductal carcinoma was the most common histological type comprising 171/178 (96.1 %) of the total cases

Conclusion: We found that Ki-67 is significantly associated with other pathological parameters including Histological grade (p-value=0.017), molecular groups (p-value=0.000) as well as ER, PgR and Her-2 immunoexpression, which is evident from the p-values i.e. 0.001, 0.000 and 0.004 respectively. Therefore, we suggest that utilization of IHC4 status i.e. ER, PgR, Her-2 and Ki-67 along with histological grade and molecular subgroup can considerably affect our clinical decisions.

Key Words: Proliferation Index, Ki-67, Breast cancer, ER & PgR

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FP-Histo-0014

INCIDENTAL FINDING OF PROSTATE CANCER IN TURP SPECIMENS: A TERTIARY CARE EXPERIENCE FROM PAKISTAN

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ABSTRACT

Objective: To identify the incidence and clinicopathologic features of prostate cancer incidentally detected in patients undergoing transurethral resection of the prostate (TURP) for benign prostatic hyperplasia (BPH), and to estimate the clinical value of pathologic review of all TURP specimens.

Study design: A retrospective pathological analysis conducted from Jan, 2007, to Jan 2017 at Liaquat National Hospital and Medical College.

Material and Methods: After excluding patients with a known diagnosis of prostate cancer prior to TURP a total of 2,386 men (ages 25-98) were identified by pathology (TURP) specimens. Yearly Incidences, tumor volume, Gleason Score, Grade, pathologic stage were recorded for all incidental prostate cancer patients.

Results: A total of 256 (10.7%) patients were found to have prostate cancer. Mean Age was 68.51±9.22 years. T1a and T1b stage Prostatic Carcinoma was found in 9.9% and 90.1% of these patients respectively. 49%(126) of these patients had a high Gleason Score (8-10) . From Jan 1, 2007 to Dec 31, 2011, 8.3% and from Jan 1, 2012 to Jan 1, 2017, 12.3% patients were found to have prostate cancer respectively, a statistical rise of 4% over a 5-year interval.

Conclusion: Our analysis concludes that a large proportion (10.7%) of patients had incidental prostate cancer and the incidence was increasing in recent years in Pakistan and in comparison, to Asian countries. In Pakistan there is a scarcity of updated national Cancer registries. The growing incidence of high Gleason scores requires keen and prompt attention. The diverse ethnic and socioeconomic background of patients propels their propensity towards loss of follow up with already limited tertiary healthcare institutes in Pakistan. This pathologic review of TURP specimens is valuable for Pakistani and Asiatic populations.

Key Words: Incidental Prostate Cancer, TURP specimens, Pakistan

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HISTOPATHOLOGY POSTER PRESENTATION ABSTRACTS

P-Histo-0001

HIGH RISK HISTOLOGIC FEATURES IN RETINOBLASTOMA: A LOCAL EXPERIENCE

Natasha Sherazi[1], Faiza Kazi[2], Mumtaz Khan[3], Irum Sohail[4]
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ABSTRACT

Objective: To determine frequency of high-risk histological features in primary enucleated eyes having retinoblastoma for deciding therapeutic strategies.

Study Design: Cross-sectional study.

Place and Duration of Study: Study was conducted in Histopathology department of Foundation University Medical College Islamabad, Shifa Trust Eye Hospital Rawalpindi and Fauji Foundation Hospital, Rawalpindi. Six months (From 25-09-2017 to 24-03-2018)

Sampling Technique: Non-probability, consecutive sampling.

Material & Methods: After approval from ethical review board, all patients fulfilling the inclusion criteria were enrolled in the study. All the demographic details of the patients including age, gender, duration since the diagnosis and family history of retinoblastoma were obtained. The specimens of retinoblastoma received form eye departments were marked. Freshly cut 3-5 microns' thick sections stained with Hematoxylin and Eosin (H & E) were examined microscopically by a team of two histopathologists (a consultant and resident histopathologist). The presence or absence of high-risk histopathological features was reported /recorded in the designed proforma.

Results: A total of 150 patients were included in the study. The mean age of the patients was found to be 2.51 ± 1.30 years. The gender distribution showed male predominance. The mean duration since diagnosis was 4.70 ± 2.81 months. Positive optic nerve involvement was found in 77 patients (51.3%) and choroid invasion was found in 25 patients (16.7%).

Conclusion: The study showed that optic nerve invasion was found in significant number of patients and was more prevalent than choroid invasion in patients with retinoblastoma. This will help to better guide our patients regarding the prognosis of the disease.

Key Words: Retinoblastoma; Histology; High risk; Children; Eye.

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P-Histo-0002

THE CURRENT DEMOGRAPHIC STATUS OF BASAL CELL CARCINOMA IN PROVINCE OF SINDH

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ABSTRACT

Objective: To determine the demographic details of basal cell carcinoma in province of Sindh.

Study Design: Cross sectional descriptive study.

Material & Methods: From a total of 20000 biopsy cases that were reported at Diagnostic & Research Laboratory, Liaquat University of Medical and Health Sciences, Hyderabad within 21 months (that is from 1-January 2017 to September 01- 2018); the histopathologically diagnosed cases of Basal cell carcinoma were included in this study irrespective of age or gender.

Results: 81 cases of basal cell carcinoma were reported in 21 months. 37 (45.67%) were males & 44 (54.32%) were females. The male to female ratio was 1: 1.5. The maximum age was 85 years while minimum age was 12 years. The common sites for occurrence of basal cell carcinoma was cheek mucosa 28 (34.57%) followed by nose 24 (29.62%), upper and lower eye lids 12 (14.81%), buttock area 4(4.46%) neck 4(4.93%), lip 3(3.70%), forehead 2 (2.46%), scalp 1(1.23%), breast 1 (1.23%) and pubic region 1(1.23%).

Conclusion: Basal Cell Carcinoma was slightly more predisposed in females. The pattern of occurrence of Basal Cell Carcinoma was more in sun exposed anatomical areas however, in our study fewer of cases were present in areas that were unexposed to sun rays.

Key Words: Basal Cell Carcinoma, sun exposure, demography.

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P-Histo-0003

MATRIX METALLOPROTEINASE 9 EXPRESSION IN SQUAMOUS CELL CARCINOMA OF HEAD & NECK; A PROGNOSTIC MARKER

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ABSTRACT

Objective: To determine the MMP 9 expression in SCC and determine if there is any relation between SCC grade and MMP 9 expression.

Study Design: Descriptive study
Material and Methods: 49 patients presenting with various histological grades of SCC were recruited & selected from Myo & Jinnah Hospital Lahore and Nishtar Hospital Multan during study period of April 2014 to December 2014. Clinical & gross examination findings were noted followed by immunohistochemical staining of SCC samples with anti MMP 9 antibody. Sections were microscopically scored for intensity (0-3) & proportion (0-3). Overall score was obtained by adding intensity & proportion scores. Tumors were categorized into low & high expression groups.
Results: Among 49 cases of SCC, n=18(36.7%) showed strong staining intensity (score 3) of MMP ,9 antibody staining in tumour cells, n=14 (28.6%) showed moderate staining intensity (score 2), n=16(32.7%) showed weak intensity &only n=1 showed negative staining. For n=3 (6.1%) cases overall expression was weak while n=46(93.9%) cases showed high expression. Statistical relation between histological grade & overall expression was found to be significant by applying Mann-Whitney U test
Conclusion: Our results clearly demonstrated that there is marked expression of MMP 9 in SCCs with a high histological grade of malignancy. In this regard, antibodies against specific MMPs or MMP blocking agents may represent helpful approaches as adjuvant in treatment for patients at the initial stages of SCC.
Key Words: Matrix metalloproteinases, Extracellular matrix, Squamous cell carcinoma

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P-Histo-0004

MORPHOLOGICAL STUDY AND DETERMINATION OF LYMPHOCYTIC INFILTRATE IN FOLLICULAR ADENOMA AND PAPILLARY CARCINOMA THYROID IN LOCAL POPULATION OF LAHORE, PAKISTAN

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ABSTRACT
Objective: To determine the frequency and clinical spectrum of Tyrosinemia type 1 in patients tested for urine Succinylacetone by gas chromatography-mass spectrometry (GCMS).
Study Design: An observational study.
Methods: **S**tudy was conducted at the Biochemical Genetic Lab (BGL), section of Chemical Pathology, Department of Pathology and Laboratory Medicine. Patients tested for urinary succinylacetone from January 2015 to October 2017 at the Biochemical Genetic Lab, AKU were included in this study. The urine samples were quantified for succinylacetone levels by GCMS. Clinical and biochemical data was collected from the structured BGL requisition forms. Data was analyzed by Microsoft Excel 2010.
Results: A total of 140 patients were tested for urine succinylacetone and 17 (12%) showed elevated succinylacetone levels. Median age was 450 days (715-202) with 9 (53%) male patients. The median urinary succinylacetone level in patients with Tyrosinemia type I was 187mmol/mol Cr (419-114), while mean ±SD AFP, SGPT levels were 8000± 696 IU/mL, and 65 ± 58.7 IU/L respectively. In patients with Tyrosinemia type-1, parents of 14 (82.3%) had consanguineous marriage. The most common symptom was failure to thrive 10 (58.8%) followed by jaundice 8 (47.0%) and fever 5 (29.4%). Hepatomegaly was found in 11 (64.7%) of the patients while urine smelt like rotten vegetables in 4 (23.5%) of patients. However, data of liver biopsy, ultrasound, C.T scan and MRI were not available.
Conclusion: Succinylacetone is a diagnostic biochemical marker for hereditary Tyrosinemia type-1 and more than one tenth of patients suspected with Tyrosinemia type-1 showed high urine succinylacetone.
Keywords: Tyrosinemia, Succinylacetone, Organic acids, Pakistan, Gas chromatography-mass spectrometry.

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P-Histo-0005

ENDOSCOPIC BIOPSY PROVEN CO-INFECTION OF HELICOBACTER PYLORI AND GIARDIA LAMBLIA IN ADULT POPULATION OF KARACHI CITY, PAKISTAN

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ABSTRACT
Objective: Worldwide, the prevalence of pathogenic bacterium Helicobacter pylori (H. pylori) and the protozoan parasite Giardia lamblia (G. lamblia) is well known. It is more common in densely populated area with poor sanitation in developing countries as compared to developed countries. Although the prevalence of these organisms is widely studied in Pakistan but our study is a unique kind in its way where we aimed to determine co-infection of H. pylori and G. lamblia in gastric and duodenal biopsies respectively.
Study design: Retrospective
Material & Methods: This study was conducted in the department of Histopathology at Dr. Tahir Laboratory, Hamdard University & Hospital, Karachi, Pakistan during January 2016 - December 2017. All the consecutive cases of gastric and duodenal biopsies from the same patient received during 2 years were reviewed. Variables including name, age, gender, site and diagnosis were recorded. The data obtained were subjected to descriptive statistical analysis using SPSS version 22.

Results: A total of 187 gastric and duodenal biopsies (males = 99/52.9%, females = 88/47.0%) (age range = 22 to 71 years) were received through 2 years of duration. Out of 187 cases, H. pylori was found in 120 (64.1%) gastric biopsies, torphozoites of G. lamblia were seen in 42 (22.4%) duodenal biopsies, co-infection of H. pylori and G. lamblia was positive in 15 (8.0%) cases whereas no infection was observed by these organisms in 10 (5.3%) cases.

Conclusion: This study concludes a high prevalence of H. pylori & G. lamblia in the population of Karachi. Moreover, the study also noted co-infection of these organisms in the study area.

Key words: H. pylori, G. lamblia, Co-infection

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P-Histo-0006

SPECTRUM OF PRENEOPLASTIC AND NEOPLASTIC LESIONS OF INTESTINE IN A TERTIARY CARE HOSPITAL OF KARACHI, PAKISTAN

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ABSTRACT

Objective: To generate the spectrum of premalignant & malignant lesions of small & large intestine received at Dow Diagnostic Research & Reference Laboratory (DDRRL).

Study Design: Cross sectional

Methodology: All the cases of preneoplastic & neoplastic lesions of small & large intestine including biopsies as well as resection specimen received at DDRRL during the period of 8 years (2009-2016) were reviewed. The variables recorded included name, age, gender, site of the lesion & diagnosis. Furthermore, the association of diagnosis was seen with other variables. A p-value of < 0.05 was considered as significant.

Results: A total of 33 cases were diagnosed as premalignant lesions of intestine consisting of adenomatous polyp = 13/39.4%, dysplasia = 12/36.4% and adenoma = 8/24.2% cases. Moreover, we also identified 15 cases of ulcerative colitis. Insignificant association of premalignant lesions was seen with age, gender & site of the lesion (p=0.073), (p=0.155), (p=0.654) respectively.

About 451 cases were diagnosed as malignant lesions of intestine which are as follows:

i. Adenocarcinoma: grade I = 64/14.2%, grade II = 260/57.6%, grade III = 99/22.0%
ii. Squamous cell carcinoma: grade I = 2/0.4%, grade II = 7/1.6%, grade III = 4/0.9%
iii. Metastatic adenocarcinoma = 11/2.4%
iv. Neuroendocrine = 4/0.9%
v. A significant association was seen between site of the tumor and diagnosis, rectum was the commonest site for adenocarcinomas (p=0.001). We also noted a significant association between age group & diagnosis, where moderately differentiated adenocarcinoma was predominantly present in young age (age group II) (p=0.001).

Conclusion: In our study, all the lesions showed male predominance with adenomatous polyp as the commonest premalignant lesion & grade II adenocarcinoma the most common malignancy of intestine.

Key words: Adenocarcinoma, adenoma, polyp

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P-Histo-0007

EPIDEMIOLOGICAL PROFILE OF SALIVARY GLAND NEOPLASMS IN THE PROVINCE OF SINDH

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ABSTRACT

Objective: To determine the incidence of salivary gland neoplasms in the province of Sindh.

Study Design: Cross- sectional Descriptive Study

Material & Methods: The histopathologically diagnosed cases of salivary gland neoplasms at Diagnostic and Research Laboratory, Liaquat University of Medical and Health Sciences, Hyderabad were included in the study which were reported during the two-year period that is from 1st July 2016 till 30th June 2018.

Results: A total of 90 cases of salivary gland neoplasms were reported. 40(44.44%) were in males and 50 (55.56%) were reported in females. The male to female ratio was 2:3. 21(23.33%) cases were reported in the age range 1-25 years with 52(57.78%), 14(15.56%) and 3(3.33%) cases being reported in the age ranges of 26-50, 51-75 and 75-90 years respectively. The commonest site was parotid gland with 66 (74.42%) of neoplasms followed by minor salivary glands with 16(17.79%), submandibular with 06(6.68%) and sublingual gland being the least common site with 01(1.11%) of neoplasms. 62 cases (68.89%) were reported from rural areas while 28 cases (31.11%) were reported from urban areas of Sindh. Among the benign neoplasms, 58 cases (64.46%) were Pleomorphic Adenoma, 04 cases (4.44%) were Warthin's Tumour and 3 cases (3.33%) of Canalicular Adenoma. Among malignant entities, 18 (20%) were Mucoepidermoid Carcinoma, 04 (4.44%) Adenoid Cystic carcinoma and 03(3.33%) Acinic Cell Carcinoma.

Conclusion: The most frequently involved anatomical site was parotid gland. Pleomorphic Adenoma was the most common benign neoplasm and mucoepidermoid carcinoma was the most common malignant neoplasm.

Key Words: Salivary gland neoplasm, Pleomorphic Adenoma, Mucoepidermoid Carcinoma, Parotid Gland.

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P-Histo-0008

DIAGNOSING SPUTUM NEGATIVE PULMONARY TUBERCULOSIS SUSPECTS BY TRANSBRONCHIAL BIOPSY AND BRONCHOALVEOLAR SMEAR

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ABSTRACT

Objective: To diagnose sputum smear negative pulmonary tuberculosis suspects by transbronchial biopsy and bronchoalveolar lavage smear keeping bronchoalveolar lavage culture as gold standard. To compare the validity of transbronchial biopsy and bronchoalveolar smear

Material & Methods: This Cross-sectional validation study was conducted at the department of histopathology, Foundation University Medical College, Islamabad and department of pulmonology and microbiology, Fauji Foundation Hospital, Rawalpindi from May 2013 to May 2014.It comprised 96 patients who underwent bronchoscopy. Transbronchial biopsy, bronchoalveolar lavage smear preparation and bronchoalveolar lavage culture were performed on specimens of all patients.

Results: Out of 96 patients only 22 patients were actually having tuberculosis confirmed by positive bronchoalveolar lavage culture, where as 74 had only clinical and radiological suspicion of tuberculosis. The mean age of patients was 43 years with a standard deviation of ±19.1. The age range was 12-80 years. The sensitivity, specificity, positive predictive value, negative predictive value and true positives of transbronchial biopsy were 68.1%, 77%, 46.8%, 89% and 15.62% while the values for bronchoalveolar lavage were 50%, 97.29%, 84.6%, 86.7% and 11.45% respectively. Thus, the diagnostic accuracy calculated for transbronchial biopsy and bronchoalveolar lavage was 75% and 13.54% respectively.

Conclusion: Diagnosing sputum smear negative pulmonary tuberculosis patients is very important for timely management of these patients and for prevention of disease spread to others. The diagnostic accuracy of transbronchial biopsy is almost 5 times more compared to bronchoalveolar lavage smear. These modalities alone have low diagnostic sensitivities but it can be increased when both tests are performed together

Key Words: Transbronchial biopsy, Bronchoalveolar lavage, Sensitivity, Specificity

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P-Histo-0009

DUCTAL VARIANT OF PROSTATE ADENOCARCINOMA HARBOR XENOTROPIC MURINE LEUKEMIA VIRUS RELATED VIRUS (XMRV) INFECTION: A NOVEL FINDING IN SUBTYPE OF PROSTATE CANCER

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ABSTRACT

Objective: Xenotropic murine leukemia virus related virus (XMRV), is the first gamma retrovirus identified a decade ago, in human tissue bearing adenocarcinoma of prostate, followed by several researches documenting little or no prevalence of XMRV in prostate cancer samples. However, the status of XMRV within subtype of prostate adenocarcinoma has not been investigated yet. In this study, we investigated the relationship between XMRV and broad-spectrum morphological entities of prostate adenocarcinoma, including acinar, ductal and other rare subtypes.

Study Design: Cross-Sectional

Methodology: The prevalence of XMRV DNA in different histological subtypes of prostate adenocarcinoma was examined after characterizing the tumors into groups, using formalin-fixed, paraffin-embedded tissue samples from newly diagnosed prostate adenocarcinomas and archival prostate cancer tissue from our XMRV case control analysis. Broad-spectrum XMRV DNA amplification was performed by end-point polymerase chain reaction, using commercially available primer set.

Results: The study included 100 patients with prostate cancer. XMRV DNA was detected in 4 of 8 (50%) ductal adenocarcinomas, exhibiting papillary and cribriform histological features. XMRV DNA was not detected in any other variant of adenocarcinoma including acinar (0/91) and mucinous carcinomas (0/1). Majority of XMRV positive cases were biologically aggressive and present cancer at an early age upon diagnosis.

Conclusion: Ductal adenocarcinomas demonstrate a significant association of XMRV DNA while other histological variants of prostate adenocarcinoma seem unrelated to XMRV infection.

Key Words: Acinar, Adenocarcinoma, Ductal, XMRV.

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P-Histo-0010

RARE CASE OF TDT POSITIVE BURKITT'S LYMPHOMA

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ABSTRACT

Burkitt's lymphoma (BL) is a highly aggressive non-Hodgkin's B- cell lymphoma. We report the case of a sporadic form of BL in a 12-year male child who presented with fever, night sweats and weight loss for two months. On

examination he had axillary lymphadenopathy and right pleural effusion. Microscopic examination of lymph node biopsy showed medium sized atypical lymphoid cells with mature chromatin, increased mitosis and apoptosis giving a starry sky pattern. Immunohistochemistry showed strong positivity for CD 20, c-myc and CD 10. Additionally, atypical cells were also positive for BCL-6, CD 43 and TdT while CD 99 and BCL-2 were negative. A staging bone marrow trephine biopsy was performed which showed morphological evidence of lymphoma involvement. Aspirate sample was also analyzed by flow cytometry which revealed a monoclonal B- lymphoid population positive for CD 20, CD 19 and CD 10. These monoclonal B-lymphoid cells were kappa light chain restricted (surface kappa positive while surface lambda negative). Pleural fluid cytology also showed involvement by lymphoma. The morphology and most of the immunoprofile is quite characteristic of BL, however TdT positivity is an unusual finding. TdT is specific for immature B and T lymphoid cells, however literature shows up to 2% of Burkitt's lymphomas, especially in leukemic phase, can express TdT. Current case is also being studied for cytogenetic analysis to confirm the presence of t (18;14).

Key Words: Burkitt's lymphoma, TdT, Non-Hodgkin's.

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P-Histo-0011

LEIOMYOMA OF THE GALLBLADDER IN A YOUNG FEMALE: A CASE REPORT

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ABSTRACT

Introduction: Leiomyoma is a benign smooth muscle tumor which can occur in any organ, but the most common forms occur in the uterus, small bowel and the esophagus. Mesenchymal tumors of the gallbladder are rare and the benign ones are extremely rare. Here we present a case of leiomyoma of the gall bladder.

Case Presentation: A 22 years old female presented with complain of abdominal pain. Ultrasound reveal gallbladder mass for which patient underwent cholecystectomy.

Grossly, a grey white lesion was identified in the gallbladder measuring 4.5 x 3.5 cm. Cut surface of the lesion was grey, white and firm exhibiting whorled appearance. Microscopically, wall of gallbladder showed a lesion composed of intersecting fascicles of spindle shaped cells. Individual neoplastic cells contained eosinophilic cytoplasm and cigar shaped nuclei. No significant mitotic activity, cytological atypia or areas of necrosis were seen in the lesion. Overlying mucosa was histologically unremarkable. Immunohistochemical stains were performed and the neoplastic cells were diffusely positive for ASMA and Desmin while immunostians CD117, CD34 and DOG-1 were negative in the neoplastic cells.

Based on these findings, it was diagnosed as Leiomyoma of the gallbladder.

Conclusion: Leiomyoma of the gallbladder is and extremely rare entity; however, it may be an under recognized. Only a few cases have been reported to date. It is important to distinguish leiomyoma from leiomyosarcoma and gastrointestinal stromal tumors in order to avoid unnecessary long term clinical follow up.

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P-Histo-0012

MIRNA IN HEPATOCELLULAR CARCINOMA: PATHOGENESIS AND THERAPEUTIC APPROACHES

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ABSTRACT

Hepatocellular carcinoma (HCC) is a worldwide disease. Because therapeutic measures are ineffective, HCC currently has a poor prognosis. (Intro) The main causes of HCCs are alcoholism, hepatitis, and metabolic syndrome. Normally hygienic studies revealed that there is lower survival rate of patients suffering with HCC. MicroRNAs (miRNAs) consist of short non-coding sequences of RNA (20 to 24 nucleotides), which post-transcriptionally regulate the expression of the protein coding genes. MicroRNAs have been proposed to be prospective therapeutic molecules and targets. For testing miRNA-based therapies, HCC is a remarkable model because it may be targeted by delivery of oligonucleotides. Current studies show a beginning for analyzing the therapeutic prospects of miRNAs or anti–miRNAs. Generally, antitumor activity of miRNAs has been observed. Recent findings have revealed that micro-R-26a, micro-R-199, micro-R-122, anti–micro-R-221, and micro-R-494 reduced tumor growth. Studies have also revealed that micro-R-34a has now entered in clinical trial. The discovery of various murine models of HCC have provided detailed characterization of the molecular processes of HCC-causing agents that also resulted in generation of novel treatment approaches

Key Words: microRNA, carcinogenesis, antitumor activity, protein coding genes

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P-Histo-0013

CORRELATION OF CD24 EXPRESSION WITH HISTOLOGICAL GRADING AND TNM STAGING OF RETINOBLASTOMA

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ABSTRACT

Objective: To determine the frequency of retinoblastoma and correlation of CD24 expression with histological grading and TNM staging.

Material and Methods: This cross-sectional study based on the analysis of ocular enucleation specimens received at the department of Pathology, BMSI, JPMC, Karachi from

1st January 2009 to 31st December 2013. Total 80 cases of retinoblastoma diagnosed on H&E, amongst 68 cases were selected for CD24 immuno staining. The remaining cases showed inadequate material and were excluded from immuno staining. The data analyzed by using SPSS version 22.

Results: The frequency of retinoblastoma in 80 cases was 2.93% in five years data. Out of 68 cases 7.35% showed G1, 11.76% G2, 26.47% G3 and 54.41% showed G4. In TNM staging 60.29% cases were in stage IV, 10.29% each were in stage III, stage II and 19.11% showed TNM stage I. CD24 immuno staining positivity was seen in majority of cases that showed G3 and G4 histology. In G3 out of 18 cases 38.88% showed moderate and 22.22% strong immuno reaction. Amongst G4, 40.54% showed moderate and 13.51% strong positivity. Variable immune staining pattern was observed according to TNM staging. Out of 13 cases in stage I, 46.15% showed moderate and 7.69% strong positivity, in stage II out of 7 cases 57.14% was negative while 42.85% showed moderate immune staining. Major fraction of stage III i-e 28.57% showed moderate and same number of cases were strong immuno staining. Most of cases in stage IV i.e. 48.78% were negative for staining while 34.14% showed moderate and 17.07% showed sever CD24 immuno staining.

Conclusion: Majority of grade 1 retinoblastoma were in TNM stage I and II and mostly were immune negative. Lymph node and distant metastatic cases were 75% in histological G4 and 25% in G3, all of them showed moderate to strong CD24 immuno expression. These results showed that CD24 expression may be a marker of poor prognosis in retinoblastoma.

No significant correlation was found between TNM stage and CD24 immuno staining due to varied pattern of staining.

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P-Histo-0014

HIGH RISK HISTOLOGIC FEATURES IN RETINOBLASTOMA: A LOCAL EXPERIENCE

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ABSTRACT

Objective: To determine frequency of high-risk histological features in primary enucleated eyes having retinoblastoma for deciding therapeutic strategies

Study Design: Cross-sectional study

Place and Duration of Study: Study was conducted in Histopathology department of Foundation University Medical College Islamabad, Shifa Trust Eye Hospital Rawalpindi and Fauji Foundation Hospital, Rawalpindi. Six months (From 25-09-2017 to 24-03-2018)

Sampling Technique: Non-probability, consecutive sampling.

Material and Methods: After approval from ethical review board, all patients fulfilling the inclusion criteria were enrolled in the study. All the demographic details of the patients including age, gender, duration since the diagnosis and family history of retinoblastoma were obtained. The specimens of retinoblastoma received form eye departments were marked. Freshly cut 3-5 microns thick sections stained with Hematoxylin and Eosin (H & E) were examined microscopically by a team of two histopathologists (a consultant and resident histopathologist). The presence or absence of high-risk histopathological features was reported /recorded in the designed proforma.

Results: A total of 150 patients were included in the study. The mean age of the patients was found to be 2.51 ± 1.30 years. The gender distribution showed male predominance. The mean duration since diagnosis was 4.70 ± 2.81 months. Positive optic nerve involvement was found in 77 patients (51.3%) and choroid invasion was found in 25 patients (16.7%).

Conclusion: The study showed that optic nerve invasion was found in significant number of patients and was more prevalent than choroid invasion in patients with retinoblastoma. This will help to better guide our patients regarding the prognosis of the disease.

Key Words: Retinoblastoma, Histology, High risk, Children, Eye

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P-Histo-0015

FREQUENCY OF EXPRESSION OF BCL6 IN DIFFUSE LARGE B CELL LYMPHOMA

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ABSTRACT

Objectives: The aim of this study was to determine the frequency of BCL6 expression in DLBCL.

Study Design: Retrospective and prospective study.

Material and Methods: The study was conducted at Department of Pathology, Pakistan Institute of Medical Sciences (PIMS), Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU) Islamabad. A total of 57 consecutive suspected cases of DLBCL diagnosed on H&E and confirmed on immunohistochemistry were selected and evaluated for expression of BCL6 .

Results: The mean age of patients was 50.1 years. Male patients were in majority in this study. The frequency of BCL6 gene in DLBCL patients was 19/57 (33.0%). It was noted that demographic features had no association with BCL6 in this study. However, proportion wise males were found more likely to have presence of BCL6 gene.

Conclusion: BCL6 immunoexpression has a fair presence in DLBCL. It can be used an adjunct tool in evaluating patients who have DLBCL. Those patients who are positive for BCL6 gene can benefit from BCL6 specific target chemotherapy.

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P-Histo-0016

PREVALENCE OF UTERINE PATHOLOGIES IN PATIENTS PRESENTING WITH ABNORMAL UTERINE BLEEDING BETWEEN 45 TO 65 YEARS OF AGE IN HYSTERECTOMY SPECIMENS FREQUENCY

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ABSTRACT

Objective: To determine the frequency of various uterine pathologies in hysterectomy specimens with clinical history of AUB

Material and Methods

Study design: Cross-sectional study

Settings: This study was conducted in the Department of Histopathology, Foundation University Medical College, Islamabad campus and Department of Gynecology, Fauji Foundation Hospital, Rawalpindi.

Duration of study: 06 months after approval of synopsis i.e 2nd Sep, 2016 to 2nd Mar, 2017

Data Collection Procedure: After approval from Ethical Research Committee (ERC) of FUMC, all the hysterectomy specimens received for histopathology at the Department of Pathology during study period and fulfilling the pre-set criteria were included in the study. Patient's data was recorded on proforma. The hysterectomy specimens were fixed in 10% neutral buffered formalin followed by grossing, processing and paraffin embedding. The slides were manually stained with Haematoxylin and Eosin by an experienced lab technician. Microscopic evaluation was done by the trainee along with two consultant histopathologists.

Results: A total of 600 patients were included according to the inclusion criteria of the study. Mean age (years) in the study was 50.91±5.76 whereas mean duration (in months) of symptoms was 6.27±2.16. Frequency of various pathologies in hysterectomy specimens was analyzed which included Leiomyoma 189 (31.5%), Adenomyosis 172 (28.7 %), Endometrial polyp 135 (22.5%), Endometrial hyperplasia 48 (8.0), Disordered proliferative endometrium 31 (5.2) followed by Endometrioid carcinoma 25 (4.2).

Conclusion: The study concludes that there were various frequencies of uterine pathologies in hysterectomy specimens. Most frequent causes of prolonged menorrhagia were Leiomyoma, Adenomyosis and Endometrial polyp in the study.

Key Words: Abnormal Uterine Bleeding, Adenomyosis, Endometrial Carcinoma and Endometrial polyps

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P-Histo-0017

LEVEL OF ERRORS, CHANGE IN DIAGNOSIS AND THEIR IMPACT ON MANAGEMENT IN CASES REFERRED FOR SECOND OPINION SHORT RUNNING TITLE: LEVEL OF ERRORS IN CASES REFERRED FOR SECOND OPINION

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Armed forces Institute of Pathology, Rawalpindi Pakistan

ABSTRACT

Objectives: To determine the frequency of change in diagnosis and level of errors in cases referred to Armed Forces Institute of Pathology for second opinion and their impact on modifications of treatment and prognosis

Study Design: Descriptive Cross-sectional study

Place and Duration of Study: Histopathology Department, Armed Forces Institute of Pathology, Rawalpindi. March to October 2017

Patients and Methods: All the cases referred for review diagnosis were tested by applying panel of immunohistochemical markers and special stains on formalin fixed paraffin embedded tissue sections as decided on morphology. Level of errors were defined as level I error: minor discrepancy with no impact on management, level II: minor discrepancy with impact on management, level III: main category remains same but there is change/confirmation of specific diagnostic entity with an impact on management and level IV: gross changes in diagnosis with significant impact on management. Level IV was further subdivided into IV a: benign misdiagnosed as malignant, IV b: malignant misdiagnosed as benign and IVc: changes in tumor subtype.

Results: A total of 100 cases where review diagnosis was changed were included in study. Minor discrepancies (level I and II) were observed in 7% (n=7/100) cases with little or no impact on the management. Most frequent discrepancy observed as Level III in 75% (n=75/100) cases. Major discrepancy (Level IV errors) was noted in 18% (n = 18/100) cases.

Conclusion: Our study results showed high discrepancy rates for Level III and IV errors on review diagnosis. The use of extended panels of immunohistochemistry markers are the most likely explanations. We encourage getting second opinion for difficult cases, whether this second opinion is in the form of intradepartmental meeting or getting review from another advanced lab.

Key Words: Discrepancy in diagnosis, Immunohisto-chemistry, Review diagnosis Second opinion.

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P-Histo-0018

OSSIFYING FIBROMYXOID TUMOR: AN UNUSUAL SOFT TISSUE TUMOR

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ABSTRACT

Ossifying fibromyxoid tumor (OFMT) is a rare soft tissue neoplasm of uncertain origin, described for the first time in 1989 by Enzinger et al. It commonly affects middle aged males with an age range of 1-83 years. Common site of origin is subcutaneous tissue of extremities. This tumor has a spectrum of histological features comprising typical, atypical and malignant subtypes. Here we report a case of this rare tumor presenting as thigh mass in 57 years old female. Histological examination revealed an encapsulated tumor with peripheral discontinuous shell of lamellar bone and central part comprising lobules of ovoid to spindled cells arranged in sheets against myxoid background. The individual tumor cells have bland round to oval nuclei, eosinophilic cytoplasm and indistinct cell borders. On Immunohistochemical evaluation, tumor was positive for CD-10 and S-100. Depending upon the characteristic histological and Immunohistochemical features, the diagnosis of ossifying fibromyxoid tumor was made. Considering the rarity of OFMT and its different histomorphological form with variable clinical behavior, it is of utmost importance to diagnose this entity precisely. Furthermore, it is stressed upon that all the cases of OFMT should be reported as to collect more data regarding this tumor which may help in knowing what is still unknown about this particular tumor. As the exact clinical behavior is still not clear, clinical follow up must be recommended after surgical excision.

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P-Histo-0019

FREQUENT OF TRIPLE NEGATIVE BREAST CANCERS IN DIFFERENT AGE GROUPS

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ABSTRACT

Objectives: Breast carcinoma is the one of most common cancer in women. Triple negative breast cancer (TNBC) is recently defined as clinical entity referring to tumors that do not express ER, PR & Her-2 receptors and show high grade morphology, poor prognosis and survival. Our study aims to determine the frequency of TNBCs in different age groups in Pakistan.

Material & Methods: It is a cross sectional study conducted at Chugtai Laboratory Lahore, on diagnosed cases of breast cancers (n=700). Prevalence of TNBC and its relative frequency in different age groups was determined.

Results: Among 700 cases with age limit of 19-84 years, total number of TNBC determined were 108 (15.4%). 13 cases were found in females of age 0-25 years, 64 cases were found between 26-50 years and 31 cases were found in ages between 51-75 years.

Conclusion: A significant proportion of TNBC cases were found to be prevalent in females with age ranging between 27-50 years

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P-Histo-0020

THE FREQUENCY OF DIFFERENT SUBTYPES OF HODGKIN'S LYMPHOMA AND THEIR ASSOCIATION WITH EPSTEIN BARR VIRUS

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ABSTRACT

Aims & Objectives: Epstein-Barr virus (EBV) is a common infection and is associated with many malignancies, including Hodgkin's lymphoma (HL), especially in developing countries like Pakistan. Our study aims to determine the frequency of different histologic subtypes of HL and their association with EBV.

Material and Methods: It is a cross sectional study conducted on diagnosed cases of HL (n=70; 53 males & 17 females). An automated immunohistochemical technique was used with an anti-EBV-LMP1 (latent membrane protein-1) antibody. Frequency of different histologic subtypes and relative frequency of EBV-LMP1 expression in different subtypes of HL was determined.

Results: Among 70 cases, the male: female ratio was 3.1:1 with an age range of 5-80 years. Out of 70 cases, 40 (57.1%) were of mixed cellularity type, followed by 25 (35.7%) cases of nodular sclerosis HL, 3 (4.3%) of lymphocyte rich HL, 2 (2.9%) of lymphocyte predominant HL and none of lymphocyte depleted HL (0%). Expression of EBV-LMP1 was seen in 44 (63%) of the total HL cases. Of individual histologic subtypes, EBV-LMP1 positivity was observed in 28/40 (70%) of mixed cellularity HL and 16/25 (64%) of nodular sclerosis HL while all cases of lymphocyte rich and lymphocyte predominant HL were negative for EBV-LMP1.

Conclusion: Mixed cellularity HL is the most common type followed by nodular sclerosis HL. EBV is found to be most commonly associated with mixed cellularity and nodular sclerosis types of HL. It might be used, in future, as a predictive marker of HL for some specific anti-EBV therapy.

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P-Histo-0021

HYPERREACTIO LUTEINALIS: AN RARE CONDITION WITH UNUSUAL PRESENTATION ASSOCIATED WITH SINGLETON PREGNANCY

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ABSTRACT

Hyperreactio Luteinalis (HL) is a rare self-limiting condition characterized by presence of multiple theca lutein cysts resulting in bilateral ovarian enlargement. There is association with multiple pregnancy and gestational trophoblastic disease. We present a case of asymptomatic Hyperreactio luteinalis, noted during cesarean section for a spontaneously conceived normal singleton pregnancy, which itself is a rare association. Bilateral ovarian cysts were found on cesarean section which were excised. Microscopic examination revealed multiple luteal cysts with associated stromal edema of ovarian parenchyma. Usual mode of treatment is conservative.

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P-Histo-0022

NEUROENDOCRINE TUMOR (CARCINOID TUMOR) ARISING IN POSTPUBERTAL TESTICULAR TERATOMA IN A 32-YEAR-OLD MALE: CASE REPORT & LITERATURE REVIEW:

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ABSTRACT

Primary neuroendocrine tumors of testis are less common accounting for <1% of all testicular neoplasms and even rare when arise in testicular teratomas. Herein, we present a case of neuroendocrine tumor arising in postpubertal teratoma in 32-year-old male who presented with complaints of abdominal swelling associated with history of undescended left testis. His serum levels of beta-HCG, AFP and LDH were elevated. Later, left orchidectomy was performed. Macroscopically, a grey white well circum-scribed tumor of 2.5x 2.0x1.8cm within testicular parenchyma is identified. Histological examination reveal a neoplasm showing organoid arrangement of round blue cells with salt and pepper chromatin, rosettes formation and occasional mitosis. Features of mature teratoma were seen in background. Immunohistochemistry reveal positivity for Synaptophysin and Chromogranin-A with Ki-67 index of 3-20% which confirmed a well differentiated neuroendocrine tumor (Grade 2) arising in postpubertal teratoma. Prognosis of these malignant transformation depend upon stage of tumor, as teratoma with malignant transformation carry excellent prognosis following radical orchidectomy if confined to testis (Stage 1). Postpubertal teratomas are considered itself malignant with the chances of metastasis at time of presentation, so there is need to screen post-pubertal patients especially with undescended testis for development of teratomas. So that earliest possible interventions could be done prior to advanced stage / metastasis for better prognosis and long-term survival.

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CHEMICAL PATHOLOGY SCIENTIFIC SESSION ABSTRACTS

SS-ChemPath-0001

NOVEL MARKERS IN OBESITY

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ABSTRACT

To review the role of established and novel obesity related biomarkers and their associations with cardiometabolic diseases.

Keeping in view the global ever rising state of obesity, vigorous research is under way to understand its Pathophysiology as a mean for disease prevention over the past decades several biomarkers have been identified that may reflect various pathophysiological pathways which link obesity and cardiometabolic diseases.

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SS-ChemPath-0002

PROCALCITONIN AS A MARKER FOR SEPTICEMIA-EXPERIENCE WITH BAHRIA HOSPITAL LAHORE

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ABSTRACT

Procalcitonin (PCT) is a 116 amino acid precursor of calcitonin. It is normally produced by the thyroid C-cells. Serum concentrations of PCT are normally <0.05 ng/ml. In circumstances of bacterial infection and particularly sepsis, PCT is produced in large quantities by many body tissues. It is detectable within 2-4 hours and peaks within 6-24 hours (as opposed to CRP which begins to rise after 12-24 hours and peaks at 48 hours). [14-16] PCT production is not impaired by neutropenia or other immunosuppressive states. PCT levels parallel the severity of the inflammatory insult, thus in more severe bacterial infection the levels are much higher.

PCT has many advantages over other biomarkers in common clinical use such as C - reactive protein (CRP), white blood cell count, tumour necrosis factor-α, etc. The PCT measurement has many advantages over older markers which include: specificity for bacterial infection, the rapidity of its rise after an insult (6 hours), the rapid decline with immune control on infection (half-life of 24 hours), excellent correlation with severity of illness and the lack of impact of anti-inflammatory and immunosuppressive states on production.

Beside this, the procalcitonin levels can be used for monitoring response to antimicrobial therapy, diagnosis of secondary inflammations post-surgery, post-organ transplant & severe trauma, diagnosis of renal involvement in paediatric urinary tract infection, determination of antibiotic treatment length in respiratory infections. Its measurement is also valuable in diagnosis, risk stratification, and monitoring of sepsis & septic shock and differentiating bacterial versus viral meningitis etc.

Decisions regarding antimicrobial therapy should NOT be based solely on procalcitonin serum levels; procalcitonin should be placed into the clinical context of each patient scenario considering the site of possible infection, the likelihood of bacterial infection, the severity of illness, and any other pertinent clinical data.

Various algorithms have been devised for PCT levels use to start antibiotic therapy in bacterial infections and follow up with its serial measurements and also for decision of stoppage of antimicrobial therapy.

AT Bahria International Hospitals, PCT serum levels are regularly measured postoperatively in patients of liver transplant (both donors and recipients) along with other parameters for early diagnosis of secondary infection. Its regular measurements have been very beneficial in follow up of such patients receiving antibiotic therapy.

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SS-ChemPath-0003

SHORT SYNACTHEN TEST NEED FOR STANDARDIZATION OF PROVOCATION TESTING

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ABSTRACT

Testing of endocrine glands function is a cornerstone in endocrine practice. Over the past 3 decades, there has been a transition from a reliance on stimulation and suppression testing to highly sensitive trophic and target hormone assays and enhanced imaging techniques. As the accuracy of laboratory assays for trophic and target hormones continue to improve, the roles for dynamic endocrine testing will continue to evolve. A complete and up-to-date provocative testing manual remains key to the accurate performance and interpretation of these tests both to make the diagnosis and to identify the cause.

The keys to successful dynamic endocrine testing are standardized protocols, expertise in performing the tests, reliable laboratory assays and interpretation by expert/ experienced pathologists. The reasons for creating a center for provocative testing included standardization of testing protocols, patient safety and convenience, and avoiding hospitalization for complicated testing protocols is essential to facilitate tests order and reports that integrate test values

with other clinical information. Decision-focused test strategies also help defined the analytic performance standards needed for optimal laboratory support of patient care.

We give report to bringing harmonization in provocative testing using synacthen stimulation testing from Karachi Chapter of Pakistan Society of Chemical Pathology. There is a need to involve regions/institute from across Pakistan for determining region and ethnic, assay based specific cut-off. There is a need to standardize and collate protocols, testing and reporting of provocative tests in laboratories performing endocrine testing in Pakistan. If consensus can be achieved for selected test strategies in a given medical practice, laboratories can help build delivery systems that should improve the likelihood of best practice occurring.

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SS-ChemPath-0004

UNIQUE RESEARCH DESIGNS FOR LABORATORY MEDICINE

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ABSTRACT

Traditionally, residents and consultants of all the disciplines of Pathology are actively involved in clinical research; rather they act as torch-bearers for research proposals and data collection for other specialists in a hospital or institute. There are certain areas, however, peculiar to laboratory medicine, which require attention of the young and senior Pathologists of all the disciplines. These studies have different formats. Research designs, methods, sample size calculations and ethical aspects of these studies will be presented. *Recently published work of the presenter in indexed journals will be flashed to exemplify such studies (except for outcome studies)*.

a. **Diagnostic Accuracy Studies**: Such studies provide the bases for establishing clinical utility of lab tests. Whenever a test is started for the diagnosis of a disease, certain parameters are to be established to provide guidelines to the clinical colleagues. Such studies carry very high utility and are published at priority.
b. **Method Validation Studies:** These are non-clinical and pure analytical studies but once again of very high utility. Laboratory specialists should feel pride in such works.
c. **Establishment of Reference Values:** Every laboratory is supposed to establish reference values of the population it is serving. Although clinical decision limits are now available for some common analytes, reference values of uncommon and rare tests have to be established by at least one laboratory in the country.
d. **Clinical Audit:** Audit reports are now considered mandatory part of a good CV. In countries with limited resources, proper audit studies should be regularly carried out and published.

Outcome Studies: These are recently introduced studies, which determine the 'value' of the tests done by the laboratories. Different from clinical audits, outcome studies determine the improvement in health care after the introduction of a lab test in terms of financial benefits and reduction in hospital stays.

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SS-ChemPath-0005

DIAGNOSTIC APPROACH TO ENDOCRINE EMERGENCY

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ABSTRACT

Majority of clinical endocrine practice is concerned with the non-acute, elective management of various perturbations of hormonal function and tumors of endocrine glands. However, because of the complex relationships between hormonal system salt and water homeostasis, carbohydrate metabolism, immune function and cardiac status. There are specific instances in which endocrine disease may be life threatening. These situations are relatively uncommon but are particularly important by virtue of their serious nature, the fact that they are generally remediable once the diagnosis has been important in elucidating many of the fundamental principles of hormonal function and homeostasis. In this presentation following endocrine disorders will be included covering clinical feature and diagnostic workup: Addisonian Crisis, Pituitary Apoplexy, Thyroid Storm, Myxedema Coma, Acute Hypercalcemia Acute Hypocalcemia, Hypoglycemia, Hyponatremia.

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SS-ChemPath-0006

CAP ACCREDITATION – THE JOURNEY

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ABSTRACT

The presentation will give a brief outline regarding the steps and processes to obtain CAP Accreditation through Laboratory Accreditation Program comprising of pre-analytical, analytical and post-analytical aspects of quality management (QM) in the laboratory. The CAP Accreditation Program improves patient safety by advancing the quality of pathology and laboratory services

through education and standard setting, and ensuring laboratories meet or exceed regulatory requirements

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SS-ChemPath-0007

SIMULATION MEDICINE & ITS APPLICATION IN TRAINING & ASSESSMENT OF LABORATORY MEDICINE

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ABSTRACT

Previously the application of technology was limited to professors uploading their slide decks to PowerPoint ™ or learning management systems like Blackboard ™. With technology now ubiquitous in our lives the young candidates who plan to take their training in laboratory medicine are already exposed to various educational technologies. However, facilitators in laboratory medicine have been slightly slow to innovate and embrace the utilization of various technologies related to simulation medicine to enhance the learning and skills development among their trainees. The methodologies of laboratory trainings need to be addressed in the current environment that is in the process of constant upgradation of hard ware, software and their embedding on different platforms. As facilitators of laboratory medicine, we need to deliberate upon the quality of educational courses and concerns of patients and trainee safety and use the features of simulation medicine for better outcomes. Facilitators in laboratory medicine need to be knowledgeable regarding new technologies in simulation medicine, how they can be used best and for which type of training and teaching. The various topics. that can be integrated in our training using simulation methodologies are: Immersion in environment workloads and work flow, use of current analytical technology and procedures, lab information system, introduction of environment stressors / distractors e.g. phone call interruptions, recreation of authentic situations: like priority and stat testing, multi-tasking. Task breadth: a continuum from specimen procurement, data entry, testing, result, decision making, pedagogical strategies: instructor supervisions, interactions and feedback, variable learning space – virtual laboratories that contribute directly to lab operations. It's emphasized here that medical laboratory science simulations are very different from the simulations in medicine and nursing. However, there will be no replacement for physicians and teachers in laboratory medicine training, but various applications of simulation medicine are essential to be understood by the facilitators to exploit the hidden potential of this modality of teaching and training for our future laboratory professionals. Simulation medicine educational technologies is taking hold and can provide the learning experiences that have never been previously imagined. It is time to gain awareness, learn about this and its implementations process. SIMU has arrived; let's learn how to use it well.

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SS-ChemPath-0008

MICROARRAY APPLICATION IN CANCER GENE PROFILING

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ABSTRACT

Molecular pathology techniques are rapidly evolving over the last three decades with multiple versatile and novel investigative procedures now mushrooming up in the market. From the time Mulllis developed PCR to today's innovative concept of next generation sequencing (NGS), there have been multiple other small-scale projects which managed to enter the clinical market and showed promise in molecular diagnostics. Microarray is a molecular hybridization technique where one can simultaneously analyzed thousands of genes/ analytes of interest on a miniaturized solid surface. The technique in real-time can provide a versatile, fast and reasonably accurate view about genome structure, expression levels and functions with a high-through put analysis. Cancer diagnostics presently rely heavily upon conventional histopathology**,** biomarkers and immunohistochemistry**.** The current diagnostic industry especially in predicting cancer type, behaviors and futuristic predication is far from maturity and microarrays along with some other molecular techniques can help. Utilizing microarrays here will one step closer to achieving personalized medicine, providing disease prediction models and a way leap forward towards cancer cure.

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SS-ChemPath-0009

STANDARDIZATION OF PATHOLOGY LABORATORIES IN PAKISTAN – WHERE WE STAND TODAY

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ABSTRACT

Over the past 07 year a slow but a steady progress has been made in the field of pathology in order to meet the requirements of the comprehensive ISO 15189:2007 standards for medical laboratories. To this date, 08 laboratories have been awarded ISO 15189:20102 accreditation for limited scope of tests. To ensure a more efficient and comprehensive system, 05 parameters have

been discussed. The foremost of these is the continued medical education (CME) for physicians to educate the physicians. Progress has also been made to educate the technologists. PAMLS and MLTAP are 02 parallel bodies playing a pivotal role in the continual professional development of MLTs. Furthermore, PNAC has been designated as the as the natural body authorized to coordinate with other regional organizations for implementation of accreditation standard. Quality assurance programs like overseas External Quality Assurance Scheme and PT providers are costly and unaffordable. Therefore, national scheme l.ike NEQAAP which is run by AFIP is extending it services towards proficiency testing while, calibration services are being provided by NPSL in Pakistan. In order to bring the laboratories in line with international standards, a two their plan which comprises of registration by a regulation body of only those laboratories that fulfill a predefined minimum criterion is the first step. To further enhance the quality level, the laboratories are encouraged to adapt international accreditation standards on voluntary basis. However, for a developing country like Pakistan, there are a numerous problem that are being faced to attain the standards for optimal health care delivery. The foremost issue is the uneven distribution of the 08 accredited laboratories in the country. Unavailability of backup instruments is also an issue in the resource poor settings.

For a standard laboratory practice, developing and strengthening the human resource system is very important. Devising a standard curriculum for MLTs is necessary as there is a lack of training opportunities in quality assurance, attitude and practice of MLTs. Government should resolve the issue of the unaffordability of PT and calibration programs. Government can help laboratories by reducing taxes and encouraging local production of costly equipments and reagents. There is a compelling need that organizations with same missions join hands and act together to provide unifying Platform to address the needs of members. Method focused and a structured effort along with government support is needed to achieve ultimate aim of accreditation.

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SS-ChemPath-0010

PAEDIATRIC REFERENCE INTERVAL-GAPS AND CHALLENGES

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ABSTRACT

An overview of Reference intervals in Pediatrics and current gaps and challenges. Establishing reference intervals can be challenging as it requires the collection of large numbers of samples from healthy individuals. This challenge is further augmented in pediatrics, where dynamic changes due to child growth and development markedly affect circulating levels of disease biomarkers.

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SS-ChemPath-0010

CHALLENGES OF MAINTAINING GOOD CLINICAL LABORATORY PRACTICES IN PERIPHERAL - 400 BEDED HOSPITAL SETTING

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ABSTRACT

Lab system strengthening is a vital prerequisite to health care delivery advances in low resource settings. Identifying challenges in areas of instruments malfunction, availability of bio medical engineer services, delays in supplies of reagents, controls and consumables, human resource management, back up method availability, temperature and power supply issues. Availability and distribution of trained staff and their rationalization to work load, communication gaps with policy makers, maintaining cold chain of supplies, problems in outsourcing of non -available tests, delays in retrieval of reports are a few major areas that needs to be improvised to lead to better health outcomes and quality working. One-year data was analyzed retrospectively from July 2017 Sep 2018 in a peripheral hospital lab to find out the gaps in the above-mentioned areas and improvements made in a few areas to make things work better. Despite the limitations the peripheral labs try to maintain good clinical lab practices in a set of minimum requirements intended to promote reliability and integrity of lab data in clinical care and research.

SS-ChemPath-0011

DISORDERS LEADING TO HYPERTENSION IN PATIENT < 45 YEARS OF AGE

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ABSTRACT

Hypertension is the highest contributor to the global burden of disease. Most of the patients with essential hypertension do not have an identifiable cause. It is important to recognize secondary causes of hypertension, as they can be identifiable and potentially curable. Secondary

hypertension occurs in a significant number of patients less than 45 years of age (approximately 10 - 15%). Leaving undiagnosed, secondary hypertension can lead to resistant hypertension, cardiovascular and renal complications, multiple specialist referrals and an unnecessary burden on healthcare system. In young patients, renal parenchymal disease, coarctation of the aorta and fibromuscular dysplasia may be considered, while adult patients should be evaluated for endocrine disorders especially primary aldosteronism, obstructive sleep apnoea and renal artery stenosis. Endocrine causes of secondary hypertension including hypothyroidism (causing elevated diastolic B.P), hyperthyroidism (causing elevated systolic B.P), hyperparathyroidism, hypercalcemia, acromegaly, pheochromocytoma and Cushing's syndrome, are less common. Conversely, primary aldosteronism occurs with sufficient frequency so as to be considered “top of the list” for secondary endocrine causes in resistant or hypokalemia-associated hypertension. Recognition and early diagnosis of secondary causes of hypertension can lead to effective treatment, good clinical outcomes and possible reversal of end-organ damage, in addition to blood pressure control.

As the prevalence of pathological conditions leading to secondary hypertension differ in various regions, and management depends on the cause. So, knowing well the frequency of common causes of secondary hypertension in our setup could be helpful in early diagnosis of disease and further management of the patient.

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CHEMICAL PATHOLOGY FREE PAPER ABSTRACTS

FP-ChemPath-0001

BIOCHEMICAL SPECTRUM OF PARATHYROID DISORDERS DIAGNOSED AT A TERTIARY CARE SETTING

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ABSTRACT

Objective: To determine clinical and biochemical pattern of parathyroid disorders in a tertiary care setting

Design: Cross sectional study

Place and Duration of the Study: Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology, September 2017 to February 2018

Methods and Material: Study was approved from Institution Review Board of AFIP. A total of 384 reported cases were included. Diagnostic biochemical tests were PTH levels, Serum total Calcium, Ionized calcium, Inorganic Phosphorus, Alkaline Phosphatase, Serum Magnesium, Total Vitamin D and urinary calcium to creatinine ratio. Non-probability convenient sampling was used after taking informed consent from patients.

Results: A total of 302 patients were diagnosed with various parathyroid disorders.

Mean age was 48 ± 19 years, 65% were males. The majority of the patients were diagnosed as a case of secondary hyperparathyroidism (81%). Frequencies of primary (4.3% in males, 2.6% in females), secondary (57.2% in males, 23.5% in females), tertiary hyperparathyroidism (1.3% in males, 0.7% in females) and hypoparathyroidism (2.3% in males, 6% in females) is present respectively. Mean serum total calcium, phosphorus, ionized calcium, magnesium and total vitamin D were 2.24±0.38 mmol/L, 1.29±0.42 mmol/L, 1.16±0.13 mmol/L , 0.87 ± 0.11 mmol/L and 51.3 ± 21.3 nmol/L respectively. Median level for ALP and intact PTH were 120 IU/l (IQR= 179-88) and 11.8 pmol/L (IQR= 30-5) respectively. Etiology of SHPT was further studied and it was seen that 72.2% patients had CKD and 20.2% had isolated Vitamin D deficiency.

Conclusion: Parathyroid disorders have significant impact on bone health. Moreover, secondary hyperparathyroidism is emerging as a major endocrine problem, especially in CKD patients and vitamin D deficient individuals. Diagnosis with a panel of biochemical tests will be the best approach.

Key Words: Parathyroid disorders; serum calcium, inorganic phosphorus, urine calcium creatinine ratio

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FP-ChemPath-0002

FREQUENCY OF PRIMARY HYPERALDOSTERONISM IN YOUNG HYPERTENSIVES IN A TERTIARY CARE SETTING OF RAWALPINDI

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ABSTRACT

Objective: Our study was aimed to determine the frequency of Primary hyperaldosteronism in young hypertensives in hospital settings of Rawalpindi.

Place and Duration of Study: Armed Forces Institute of Pathology (AFIP) Rawalpindi, Department of Chemical Pathology & Endocrinology from June 2016 to May 2017.

Study design: Cross sectional study

Material and Methods: Two hundred and fifty patients with hypertension (blood pressure of more than 140/90 mm Hg) of both genders, with age between 17-40 years were recruited in the study. Patients on anti-hypertensive medications, renal function derangement, pregnant females and those labeled with secondary hypertension were excluded. Blood sample was taken for the analysis of plasma renin, aldosterone, electrolytes and blood gases. Parametric quantitative variables were presented as mean±SD.

Result: Eight cases out of a total 80 subjects fulfilling the inclusion criteria were diagnosed with Primary hyper-aldosteronism and 72 with Essential hypertension. Mean age of patients having Primary hyperaldosteronism was 29.25 ± 7.1 years. The mean diastolic blood pressure of all patients was 90.3 ± 6.5 mm of Hg while mean systolic blood pressure was 142.7 ± 10.5 mm of Hg.

Conclusion: Frequency of Primary hyperaldosteronism was found to be 10% emphasizing on the fact that it is not very uncommon in young hypertensives.

Key Words: Renin Angiotensin Aldosterone disorders, Primary hyperaldosteronism, Essential hypertension.

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FP-ChemPath-0003

BIOCHEMISTRY AND ENVIRONMENTAL RISK FACTORS ANALYSIS FOR PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): A PROSPECTIVE STUDY OF CHILDREN HOSPITAL, LAHORE, PAKISTAN

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ABSTRACT

Objective: The prevalence of child ALL varies from 0.9-4.7 per 100,000 children per year around the world. In Pakistan, the mortality rate of child ALL is highest (71%) in

the world. We investigated variations in blood parameters, biochemistry, distribution of ALL type and environmental risk factors in pediatric patients of Children Hospital, Lahore.

Study Design: Fifty patients (n=50) diagnosed with ALL were enrolled according to the inclusion and exclusion criteria from Children's Hospital, Lahore. Child ALL patients with infections were not included in the research.

Material and Methods: The blood samples were collected from pediatric ALL patients by taking informed consents for laboratory tests. Clinical history were collected after collaboration with the relevant departments. The information on age, gender, blood related parameters, i.e., HGB, WBCs, PLTs, biochemical parameters, i.e., ALT, AST, ALP, uric acid, creatinine. The distribution of type of ALL (B-lymphocytic acute lymphoblastic leukemia/T-lymphocytic acute lymphoblastic leukemia ALL) was determined through Immunophenotyping analysis. Information on other/environmental risk factors, i.e., socioeconomic status (poor, middle class, rich), consanguinity (presence or absence), drinking water conditions (filter/non-filter) and locality (urban/ rural) were also collected on a designated Proforma. Chi Square (χ^2) tests were applied to look a relative significance at p<0.050 in all of the mentioned parameters between normal and disturbed values out of total.

Results: The ALL was most common in children at 1–5 years of age (90%) and in male patients (62%). The risk factors which were disturbed were: the drinking water (non-filtered, 62%), socioeconomic status (middle class, 58%), consanguinity (present, 54%) and locality (rural, 64%). The parameters which were disturbed, included the values of PLTs-platelets (92%), HGB-hemoglobin (88%), WBCs-white blood cells (72%) and creatinine (86%). While, biochemical parameters: ALT, AST, ALP and uric acid did not show any significant variations. The occurrence of B-ALL was most frequent (92%) as compared to T-ALL. Chi square test showed a significant difference (p=0.000<0.050) between socioeconomic statuses: poor, middle classes & rich, filtered & unfiltered water intake, positive & negative consanguinity, rural & urban locations, and the type of ALL (B-ALL or T-ALL). Chi square test showed a significant difference (p=0.000<0.050) between normal and disturbed levels of hemoglobin, WBCs and PLTs, out of total. The levels of ALT, AST, ALP and uric acid were normal in all patients.

Conclusion: Important environmental risk factors for child ALL incidence were linked with unfiltered drinking water, middle-class status and rural location. Other risks in children were related to B-ALL, consanguinity (present), male gender, age less than 5 years. Variations in complete blood count (CBC) parameters were evident, but there were insignificant variations in liver enzymes and uric acid levels.

Key Words: Acute Lymphoblastic Leukemia (ALL), type of ALL, Environmental Risk Factors, Biochemistry.

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FP-ChemPath-0004

CYSTIC FIBROSIS SCREENING: DOES A NEGATIVE SWEAT CONDUCTIVITY TEST RULE OUT CYSTIC FIBROSIS?

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ABSTRACT

Objective: To present clinicopathological features and treatment outcome of 8 patients with anti NMDA receptor antibody mediated encephalitis with a view to document evidence to facilitate timely diagnosis and recommend specific therapy.

Type of study: Case series

Place and Duration of Study: December 2016 to February 2018, Shifa International Hospital. Inclusion criteria was patients with anti NMDA receptor antibody Encephalitis. Exclusion criteria was patients with any other psychiatric illness.

Results: Eight patients tested positive out of a total of 247 specimens received with suspicion of anti NMDA receptor antibody encephalitis. There were 7 females and 1 male with a mean age of 15 years and age ranging from 1 year to 28 years at presentation. Prodrome with fever and flu like illness was observed in 5 patients, seizures in 8 patients, and memory deficit in 4 patients, delusions and paranoia in 2 patients, hallucinations were documented in 1 patient. The youngest 3 patients presented with hyperactivity and irritability along with seizures. Five patients responded well to a combination of steroids and IVIg and showed complete recovery. One patient received steroids, followed by plasmapheresis and IVIg and recovered completely. Two patients were treated with steroids only and suffered residual speech and motor deficit.

Conclusion: Anti NMDA receptor antibody was found in about 3.2% of specimens referred for this test. Anti NMDA receptor antibody encephalitis should be suspected in young females presenting with seizures, memory deficit, delusions, paranoia, hallucinations and altered consciousness. Children present with hyperactivity, irritability, seizures and altered consciousness. Patients who are treated with combination immunosuppressive therapy including steroids, IVIg and plasmapheresis have a high probability of complete recovery.

Key Words: Anti NMDA receptor Encephalitis, Immunosuppressive therapy, steroids, plasmapheresis, IVIg.

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FP-ChemPath-0005

ASSOCIATION OF ENDOCRINOPATHIES WITH CHRONIC KIDNEY DISEASE

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ABSTRACT

Objective: To assess the pattern of endocrine abnormalities in patients with chronic kidney disease.

Study design: Cross sectional

Material and Methods: A total of 95 subjects were recruited in the study, of which 71 cases (34 CKD patients who were not on dialysis while 37 dialysis patients) and 23 controls were included in the study. Patients with history of any endocrine disorder were excluded from the sample. Routine chemistry and hormonal profile were assayed along with blood complete picture.

Results: Our sample had 57 males, and 38 females. Mean age of the sample was 46.5 (47.3 in males and 45.3 in females) while amongst other comorbidities, 44 were hypertensive and 17 were diabetics while no other comorbidity was noted in the remaining. Hemoglobin levels (p=<0.001, r=0.392) platelet (p=<0.001, r=0.46) and red cell count was found to have strong association and weak positive correlation with declining eGFR. TSH and FSH was found to be strongly associated with eGFR in females with a moderate negative correlation while prolactin was found to be having strong association but weak and positive correlation with eGFR. While in males none of the hormones assayed showed strong association with eGFR except T4, which showed a moderate association with moderate positive correlation with eGFR.

Conclusion: Our study showed that eGFR has a stronger association with endocrinopathies in females as compared to males.

Key Words: Endocrinopathies, chronic kidney disease, hormonal imbalance.

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FP-ChemPath-0006

TO DETERMINE CUTOFF VALUE OF TRIGLYCERIDES TO HDL RATIO IN CARDIO VASCULAR RISK FACTORS

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ABSTRACT

Objective: This study was conducted with the aim of determining the cutoff value for Triglycerides to HDL ratio in adults with cardiovascular risk factors.

Study design: Cross sectional study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology Rawalpindi from January 2018-June 2018.

Material and Methods: This study was a cross-sectional study conducted after the Institutional Review Board's (IRB) approval at Armed Forces Institute of Pathology from January 2018- June 2018. Data was collected from 354 patients. Inclusion criteria included adults with 19-50 years of age. Patient with comorbidity like cancer, tuberculosis, bed ridden patients were excluded from study. Sampling technique was simple random sampling which was done by simply picking participants through random numbers.

Results: Out of a total 355 patients selected, 269(71.5%) were females while 86(22.9%) were males with mean age of 37±11.64 years with range of 22-60 years of age group. A cut off of 1.0 for Triglycerides to HDL–c ratio was able to identify participants with cardio metabolic risk factors (obesity, hypertension, diabetes). The AUC of ROC for the ability of TG/HDL-C ratio to predict cardio metabolic risk factors was significant with coordinates of 0.68±1.60 (p-value = 0.03). Particularly with a cutoff point of 1.0 it showed a sensitivity of 76% while specificity was 64% for early diagnosis of cardiovascular risks factors.

Conclusion: This study concluded that using 1.0 as an optimal cutoff of TG/HDL ratio can be used as a predictor and an early marker for cardio metabolic risk factors.

Key Words: High density lipoprotein, cardiovascular disease, TG/HDL Ratio, Obesity.

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FP-ChemPath-0007

ASSOCIATION OF ALPHA PROTEIN LEVELS WITH THE VIRAL DISEASE IN PATIENTS WITH HBV AND HCV INFECTION

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ABSTRACT

Objective: To determine association of alpha-fetoprotein (AFP) levels with presence and severity of disease in patients with hepatitis B and hepatitis C.

Study Setting and Duration: The study was carried out in Pathology Department, Sheikh Zayed Hospital and Medical College, Rahim Yar Khan from between 2016 and 2018

Material and Methods: The study was based on analysis of clinical and laboratory data collected, as part of the routine management of patients with CLD in our hospital. Records of 230 consecutive subjects, 145 males and 85 females were analyzed. They were patients with biopsy proven chronic liver disease (CLD) referred to the Pathology Departments of the hospital for serum AFP, liver function tests, Hepatitis B surface Antigen (HBsAg) and anti-hepatitis C antibody (Anti-HCV) investigations and viral load by RT-PCR. Their health records were searched for diagnosis, age, sex and other medical history of the patients at the time of presentation. A specific laboratory code was assigned for each patient so that no name or identifier other than age, sex appears in our record. AFP level was assayed using ELECSYS e411 by Roche diagnostics, Switzerland. The principle of the technique is based on Electrochemiluminescence immunoassay. HBV and HCV viral load was detected using quantitative Real time RT-PCR. While the liver functions tests were

evaluated using Beckman Coulter AU-480 USA. Twenty-five males and 25 females of similar ages were used as controls. They were recruited in 2017 from disease-free staff and students of the hospital, who had no evidence of CLD based on history and normal laboratory findings. Students-*t*-test for parametric data was used for statistical comparison of the results. Pearson`s Correlation was used to study the correlation between AFP levels and viral load.

Results: There were 145 male and 85 females with age ranged from 30 to 70 with a mean of 43± 6.5 years. Hundred (43%) out of 230 subjects were seropositive for HbsAg while 130 (57%) were seropositive for Anti HCV. The control subjects were negative for both HBsAg and Anti HCV.The mean AFP level of the study patients was 159± 9.9 ng/mL while the mean of control was 1.93 ± 0.24 ng/mL ($p < 0.01$). The mean (AST) activity was 145 ± 1.8 u/L, while the mean control value was 10.8 ± 1.2 u/L. The means (ALT)and alkaline phosphatase (ALP) activities were 75 ± 5.1 u/L and 176 ± 1.6 u/L, respectively, while the means activities in control subjects were 8.6 ± 1.2 u/L and 29±4.2u/L, respectively. The means total bilirubin and direct bilirubin were 68.7 ± 6.8 µmol/L and 26.0 ± 4.1 µmol/L, while the means of the control subjects were 10.6 ± 1.2 µmol/L and 4.2 ± 1.0 µmol/L, respectively. The means total protein, albumin and globulin were 50.6 ± 3.6 g/l, 29.6 ± 1.9g/L and 20.6 ± 2.8 g/L, respectively, their mean control values were 65.2 ± 1.1 g/L, 42.1 ± 1.1 g/L and 21.9 ± 1.2 g/L. Statistically significant differences were observed in all the parameters ($P < 0.001$) except for globulins. No correlation was found between AFP and viral load of hepatitis B and hepatitis C.

Conclusion: Serum AFP level was highest in CLD patients with HCV antibody positive compared to HBsAg positive CLD and hepatitis negative patients but AFP cannot be used as a marker for quantitative estimation of viral load. A high level of suspicion of HCV infection should be maintained when high levels of AFP is recorded in patients with CLD.

Key Words: Chronic hepatitis, HCV, HBV; Alpha-fetoprotein; Viral load.

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FP-ChemPath-0008

EVALUATION OF CYSTATIN C AS BIOMARKER OF GESTATIONAL DIABETES MELLITUS

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ABSTRACT

Objective: To evaluate the Cystatin C as screening biomarker for gestational diabetes mellitus (GDM).

Study Design: Case Control

Material and Methods: This study was conducted at department of chemical pathology Army Medical College and Military Hospital Rawalpindi. A total of 30 women with gestational diabetes (cases) and 30 healthy pregnant women (control) were recruited in the study. HbA1C, cystatin C and insulin levels were performed on samples of all the participants. Seventy-five-gram OGGT was performed on all subjects. Paired T-test and Odds ratio were calculated for cases and controls.

Results: The cystatin C levels were high with increasing parity. Paired sample t test showed strong association of cystatin C to HbA1c, fasting and post load glucose levels in patients with GDM (HbA1C t (60) =36.0, $p<0.001$, Fasting t (60) =34.3, $p<0.001$, One-hour t (60) =27.6, $p<0.001$ and Two hours t (60) =22.9, $p<0.001$). Adjusted odds ratios (OR) based on cut off value of cystatin C (> 0.95) of maternal plasma showed positive association with one-hour (OR=4.7) and two-hour (OR= 4.3) post load plasma glucose levels in OGTT.

Conclusion: Based on results of our study, Cystatin C may be used as preliminary screening biomarker for GDM. Only those patients having elevated levels of Cystatin C can be further evaluated using OGTT and once diagnosed appropriate management strategies may be instituted

Key Words: Cystatin C, Gestational diabetes.

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FP-ChemPath-0009

BMI AND OTHER LIFE ASSOCIATED RISK FACTORS AMONG APPARENTLY HEALTHY MEDICAL STUDENTS

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ABSTRACT

Objective: To find the prevalence and perception of BMI and other lifestyle risk factors among apparently medical students in tertiary care hospital, Karachi

Study Design: This is a cross sectional study to find the prevalence and perception of lifestyle risk factors among apparently healthy medical students in tertiary care hospital, Karachi

Material and Methods: A questioners addressing the life style habits was administered to determine the age, gender, physical activity, and eating habits. Height and weight will be noted to calculate BMI. The specific scores were given to responses of various questions, and data was analyzed using SPSS (Release 13.0, standard version, copy right).

Result: Three forty-seven students aged 18–25 years completed a self-reported questionnaire. Around 52.6% of the participants were consuming. fast foods at least once a week. walk of >20 min was being practice by 13% of students only. Males and females had a high prevalence of being overweight and obesity and low levels of PA. In this study BMI was found to be significantly different between: gender (P-value <0.001), socioeconomic status (P-value <0.001), study stream (P-value <0.001) year of study (P-value<0.001), soft drink non-user's vs twice weekly users (P-value 0.042), weight trainer's vs no sport (P-value

0.033) and addiction of cigarette and pan chalia vs non-addiction (P-value0.004).

Conclusion: This study reveal that BMI IS significantly affected by various risk factors. Thus, it can be concluded that only knowledge and increasing perception is not sufficient to prevent the onset of lifestyle diseases among apparently healthy literate medical students. This is a pilot study which would make a basis for large scale longitudinal study to intervene and prevent life style risk factors among future asset of practicing practitioner.

Key Words: Lifestyle diseases, eating habits, Physical activity.

FP-Chem Path -0010

EFFECTS OF Δ-TOCOTRIENOL ON MODULATION OF GLYCEMIC CONTROL & INFLAMMATORY BIOMARKERS IN PATIENTS WITH DIABETES MELLITUS

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ABSTRACT

Objective: Diabetes mellitus is a global health problem and associated with Inflammation, increased oxidative stress and impaired insulin action. δ-Tocotrienol being potent anti-oxidative and anti-inflammatory agent, has shown beneficial effects in different chronic diseases. The objective of this study is to find out the effect of delta tocotrienol (250 mg) supplementation along with recommended diabetic medications on fasting glucose, glycated Hb, total cholesterol, LDL-C, and hs C-reactive protein (hs-CRP) in patients of type 2 diabetes mellitus.

Study Design: Randomized placebo-controlled double blinded prospective clinical trial.

Material and Methods: In this ongoing study; 60 diagnosed patients of type-2 diabetes mellitus aged ≥30 years of either sex with fasting glucose 7-12 mmol/L and HbA1c ≥ 6.5-11% were included. Persons having history of acute illness, liver, renal, thyroid disorders or malignancy and history of taking anti-inflammatory drugs, vitamin E were excluded from study. Patients were grouped into two; 30 patients in group A and 30 in group B by a simple random draw. Subjects in the group A were given capsules containing 90% pure δ-tocotrienol 125mg twice daily and group B was provided placebo twice daily for four months. No adverse effect was reported. Total 5 ml blood was collected in EDTA and plain tubes for analysis of biochemical markers at start and after 16 weeks of study. Statistical analysis was done on SPSS- 2. Statistical significance was set at p< 0.05.

Results: δ-Tocotrienol supplementation led to significant reduction in the pre vs post levels of biomarkers including fasting glucose, HbA1c, total cholesterol, LDL-C and hs-CRP in tocotrienol group were 7.18±1.95 vs 6.30±1.70 mmol/L, 8.32±1.09 vs 7.36±1.21%, 5.69±0.74 vs 4.63±0.82 mmol/L, 3.77±1.35 vs 2.81±0.93 mmol/L and 3.51±1.95 vs 2.62±1.50 mg/L respectively (P<0.05). The serum triglyceride did not change significantly compared with the baseline measurements. However, there was no significant change in the, pre vs post levels of the biomarkers in the placebo group(p=NS).

Conclusion: Delta-Tocotrienol demonstrated significant improvement in glycaemic control, lipid profile and reduction in serum hs CRP in the patients with type 2 diabetes mellitus. The δ-tocotrienol supplementation in addition to antidiabetic drugs at early phases of the disease can be helpful in the prevention of long-term diabetic complications.

Key Words: Diabetes mellitus, δ-tocotrienol, Fasting glucose, Glycated Hb, hs-CRP.

FP-ChemPath-0011

THE DETERMINATION OF MEDIANS OF BIOCHEMICAL MATERNAL SERUM MARKERS IN HEALTHY WOMEN GIVING BIRTH TO NORMAL BABIES

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ABSTRACT

Objective: To determine median values of biochemical maternal serum markers in local population during second trimester maternal screening to rule out most common chromosomal anomalies, Down syndrome.

Study Design: Cross sectional study for estimation of reference values.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), November 2016 - May 2017.

Material and Methods: Non-probability consecutive samplings, all healthy pregnant women with single pregnancy, were included. As non-parametric statistics was used, the minimum sample size is 120. Blood sample for serum Human chorionic gonadotropin (HCG) was analyzed on Random access immulite 2000®, Alpha-fetoprotein (AFP) was analyzed on ADVIA Centaur®, Unconjugated Estriol and Inhibin A measured by enzyme-linked immunosorbent assay method by PR 4100 Micro plate Reader®.

Results: Total 155 women were enrolled into this study. The age of all women enrolled ranged from 30 to39 yrs. Among them, 39 per cent of women were less than 34 years. Mean maternal age 33.46±2.35 SD and maternal body weight were 54.98±2.88. Median value of quadruple markers calculated from 15-18th week of gestation that will be used for calculation of MOM for screening of trisomy21 in this gestational age. Median value at 15 week of gestation were observed HCG 36650 mIU/ml, AFP 23.3 IU/ml, UE3 3.5 nmol/l, Inhibin-A 198 ng/l, at 16 week of gestation HCG 29050 mIU/ml, AFP 35.4 IU/ml, UE3 4.1 nmol/l, Inhibin-A 179 ng/l, at 17 week of gestation HCG 28450 mIU/ml, AFP 36.0 IU/ml, UE3 6.7 nmol/l, Inhibin-A 175 ng/l and at 18 week of gestation HCG 25200 mIU/ml, AFP 38.2 IU/ml, UE3 8.2 nmol/l, Inhibin-A 190 ng/l

respectively. All the comparisons were significant (p-Value <0.005) with 95% confidence Interval (CI) and level of significance of study set by going through literature and set at 5%.

Conclusion: The median values for these four biomarkers in Pakistani pregnant women can be used to calculate MoM.

Key Words: Down syndrome, Median, Quadruple test, Screening, Serum biomarker, Second trimester

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FP-ChemPath-0012

DIAGNOSTIC ACCURACY OF CANNABINOID TESTING BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY IN HUMAN HAIR

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ABSTRACT

Objective: To determine the diagnostic accuracy of Cannabinoids testing by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS) in human hair and to compare it with urine for detection of cannabis use in civil heavy vehicle drivers.

Study Design: Diagnostic accuracy (validation) study

Place and duration of study: This study was carried out at Department of Forensic Medical Sciences Laboratory (FMSL), Armed Forces Institute of Pathology Rawalpindi, Pakistan from February to November 2017

Materials and Methods: Hair and urine samples of 151 civil heavy vehicle drivers were collected from various areas of Punjab. Sampling technique was non-probability convenient. About 10 ml of urine was collected from each subject and stored at -20 °C. Hair strands were and collected kept at room temperature. Separation of compounds was done on Agilent Poroshell and analyzed on a 6460 Triple Quadrapole LC-MS along with software Mass hunter ©.

Results: All the 151-male civil heavy vehicle drivers, who were included in the study, were categorized into three main groups. There were 69(45.7%) truck drivers,43(28.5 %) twenty-wheeler drivers and 39(25.8%) bus drivers. Mean age was 36±10.82 years. Paired t-test was applied to check mean difference between the two tests' concentration (i.e. urine and hair analysis for cannabis) which shows significant difference at **p<0.001**. Various parameters of diagnostic accuracy in hair and urine samples were: Sensitivity (96% and 62%), Specificity (93% and 95%) Positive Predictive Value (88% and 87%), Negative Predictive Value (97% and 82%) respectively. Overall diagnostic accuracy of Cannabinoids in hair was 94% while in urine it was 83%. Receiving Operating Characteristics (ROC) curve was plotted which showed area under curve of 0.96 and 0.79 for hair and urine respectively.

Conclusion: This study highlights the importance of hair as an alternative biological matrix due to its good diagnostic yield, non - invasive specimen collection and analyte stability, as well as wider detection period compared to urine.

Key Words: Cannabinoids testing in hair, liquid chromatography-tandem mass spectrometry (LC-MS/MS), diagnostic accuracy.

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FP-ChemPath-0013

EFFECT OF PRE-ANALYTICAL VARIABLES ON SERUM TSH BY CHEMILUMINESCENCE METHOD

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ABSTRACT

Objective: Our study was aimed to identify effect of pre-analytical variables on serum thyroid stimulating hormone.

Place and duration of study: Armed Forces Institute of Pathology (AFIP) Rawalpindi, Department of Chemical Pathology & Endocrinology from March 2018 to August 2018.

Study design: Cross sectional study

Material and Methods: Hundred subjects with ages ranging from 18 to 34 years, irrespective of gender were randomly selected for this study. Five milliliters venous blood sample was collected from each subject in a serum separator and divided into two aliquots. First aliquot was centrifuged and analyzed immediately for TSH, while second aliquot was stored for 24 hours and was then analyzed. TSH was measured by third generation assay using chemiluminescence technique on ADVIA Centaur® XP. Serum TSH levels were also analyzed twice daily; in the morning (0800 to 0900 hours) and afternoon (1400 to 1600 hours). Data was analyzed using SPSS version 24. Frequency and percentages were calculated for qualitative variables like gender and pre-analytical variables. Test of significance, Mann Whitney t test was applied and p value <0.05 was taken as significant.

Result: Mean age of subjects was 23 ± 3.4 years. Change in circadian rhythm was observed in 28% (17) males and 36% (14) females. Statistically significant association was found between morning and evening TSH levels, while no change was observed in TSH level by early and late centrifugation of samples.

Conclusion: TSH levels vary significantly between blood samples collected at different times of the day from the same person. TSH is resistant to degradation, immunologically stable, and reasonably insensitive to potential problems associated with routine specimen handling, when measured by immunoassay technique. Therefore, it is helpful in large epidemiological studies and small size laboratory, which require long transportation time and storage.

Key Words: Thyroid stimulating hormone, Preanalytical Variables, Centrifugation.

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FP-ChemPath-0014

EVALUATION OF A NOVEL CLINICO-BIOCHEMICAL SCORE FOR SCREENING OF INHERITED METABOLIC DISEASES IN PEDIATRIC POPULATION

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ABSTRACT

Objective: To evaluate a Novel Clinico-Biochemical Score for Screening of inherited metabolic diseases (IMDs) in Pediatric Population in our setup

Study Design: Diagnostic Accuracy (validation) Study.

Place and duration of study: Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, August 2016 to August 2017.

Material and Methods: We collected and evaluated clinical data, preliminary biochemical investigations, plasma amino acid (PAA)and organic acid profiles (where indicated) of 354 children, aged < 1 year to 12 years, who were referred to our department for evaluation of suspected Inherited Metabolic Diseases, from August 2016 to August 2017. A clinico-biochemical score card named ***R***awalpindi ***I***nherited Metabolic Diseases ***Sc***ore (RISc) was devised, on a scale from 1 to 10, incorporating 5 clinical and 5 important biochemical findings, and each variable was assigned a "score" based on it's relative frequency/risk. Each case was then assigned the RISc score and evaluated for presence or absence of any Inherited Metabolic Disease based on the score. This score was validated keeping Plasma amino acids and organic acid profiles (in selected cases) as reference standard.

Results: Patients were divided into three groups based on RISc score as low RISc (0.5-2.5), medium RISc (3.0-5.5) and high RISc (6-10). A total of 354 cases reported in2016 and 2017 and 33(9.3%) were diagnosed to be having IMDs. 1(3.0%) patient from low RISc, 4(12.1%) from medium RISc and 28 (84.8%) from high RISc group were found to test positive for any one IMD. High RISc group had a statistically significant higher IMD rate than the other two groups ($p<0.001$). Specificity, sensitivity, positive likelihood ratio; negative likelihood ratio, positive predictive value, negative predictive values and accuracy were 93%,85%,11.8, 0.16, 55%, 98% and 90%, respectively.

Conclusion: The cost effective RISc card based on clinical data and preliminary biochemical investigations is a breakthrough in diagnosing IMDs in cost restrained setups. It is strongly suggested that the initial screening for suspected IMDs and decision for advanced laboratory testing be carried out based on the RISc card presented in the study.

Key Words: Inherited metabolic disease, Plasma amino acids, Organic acids, Rawalpindi Inherited Metabolic diseases score.

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FP-ChemPath-0015

TO DETERMINE THE FERTILITY PROFILE OF AIS PATIENT IN PAKISTANI POPULATION

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ABSTRACT

Objective: Current study was aimed to determine population based cut off of fertility profile-serum LH, FSH, testosterone and dihydrotestosterone in patients with AIS presented at different ages at tertiary care hospital of Rawalpindi, Pakistan.

Study design: Cross sectional study

Place and duration of study: Department of Chemical Pathology & Endocrinology Armed Forces Institute of Pathology (AFIP) Rawalpindi, from Jan 2016 to Dec 2017

Methods and materials: Ninety-one (91) patients, diagnosed as cases of AIS, were included in the study. Subjects were consecutively selected as per inclusion and exclusion criteria. Blood samples were collected from each subject for basal serum testosterone, serum luteinizing hormone (LH) and serum follicular stimulating hormone (FSH) level. Human Chorionic Gonadotropins (hCG) stimulation test was performed in each subject as per laid down protocol. Sandwich chemiluminescence immunoassay technique was used to analyze serum testosterone, LH and FSH. Serum dihydrotestosterone was also analyzed to calculate testosterone and dihydrotestosterone (T/DHT) ratio.

Result: Mean age of subjects was 1.78 +/-0.95 years. Cut off of different analytes were:: serum LH (IU/L) 6.80 (in 1 day to 1 year of patient), 6.74 (1 year to 10 years) 6.6 (>10 years):serum FSH (IU/L) 9.71 (1 day to 1 year), 9.01 (1 year to 10 years), 10.01 (>10 years): serum testosterone before hCG stimulation test (ng/dl) 107.32 (1 day to 1 year), 120.76 (1 year to 10 years), 98.32 (>10 years) and 310.39 (1 day to 1 year), 354.71 (1 year to 10 years), 293.43 (>10 years) after hCG stimulation test. Similarly serum dihydrotestosterone (pg/nl) were 22.72 (1 day to 1 year), 26.32 (1 year to 10 years), 21.59 (>10 years) and T/DHT levels were 13.65 (1 day to 1 year), 13.99 (1 year to 10 years) and 13.84 (>10 years) respectively. Patients were diagnosed having AIS on basis of hCG stimulation response, with serum testosterone 2 to 9 times of basal level.

Conclusion: In this population-based study, we concluded that cut off of fertility profile of patients of AIS varies according to age and these cut off are quite helpful in diagnosis of patient with AIS.

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FP-ChemPath-0016

COMPARATIVE EXPRESSION PROFILING OF SERUM MICRORNA-21 IN HEPATITIS B VIRUS-ASSOCIATED CHRONIC HEPATITIS, LIVER CIRRHOSIS AND HEPATOCELLULAR CARCINOMA PATIENTS

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ABSTRACT

MicroRNAs (miRNAs) represents a class of evolutionary conserved non-coding small RNAs that have emerged as master regulators of several biological processes. In the present study, we report the comparative expression profile of serum miR-21 in 72 patients with chronic hepatitis B (CHB), 64 with HBV-associated liver cirrhosis (HBV-LC), 57 patients with HBV-associated hepatocellular carcinoma (HBV-HCC) and 60 healthy controls that were prospectively enrolled. Total RNA was extracted from patients of each group as well as that of healthy controls and miR-21 levels were analyzed using qPCR.

Our results demonstrated that the expression of miR-21 was significantly up-regulated in serum samples of CHB, HBV-HCC patients ($P < 0.01$) in comparison to levels of serum miR-21 from healthy subjects. On the other hand, serum miR-21 levels were significantly downregulated ($P < 0.05$) in HBV-LC patients. Receiver operator curve (ROC) analyses showed that the serum miR-21 levels can serve as marker for discriminating patients with CHB, HBV-LC and those with HBV-HCC from healthy controls where the maximum sensitivity and specificity ratio of serum miR-21 was observed in case of HBV-HCC versus healthy controls. Moreover, serum miR-21 levels showed superior sensitivity: specificity ratios in comparison to that of AFP levels in discriminating HBV-HCC patients.

In summary, serum miR-21 has the potential to serve as a non-invasive biomarker for HBV-associated liver damage and disease progression.

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CHEMICAL PATHOLOGY POSTER PRESENTATION ABSTRACTS

P-ChemPath-0001

LYSINE PROTEIN INTOLERANCE - CLINICOPATHOLOGICAL SPECTRUM OF A RARE DISEASE

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ABSTRACT

Objective: To assess clinicopathological characteristics of LPI from cases reported at the biochemical genetic laboratory of AKUH.

Study Design: Retrospective observational study

Methods: This study was conducted at BGL in the Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, AKUH from January 2013 - February 2017. A team consisting of a Chemical pathologist and a resident reviewed Plasma and urine amino acids (AA) reports of patients reported as LPI and clinical details were recorded form a structured pre-filled questionnaire. SPSS version 21 was used to analyze the data. Frequencies and percentages were calculated for gender, consanguinity, and clinical presentations, biochemical features.

Results: We identified 6 patients, all were male with age range of 10 days old to 24 months. Five patients had decreased levels of lysine, ornithine and arginine in plasma whereas only 2 patients had elevated levels of these amino acids in urine samples. History of consanguineous marriage was found in all six patients. In all six patients, vomiting was the most common symptom followed by seizures, developmental delay, and drowsiness. Hepatomegaly was present in all patients.

Conclusion: Lysine protein intolerance is a rare metabolic disease resulting from recessive inherited mutations in the *SLC7A7* gene encoding the cationic amino-acids transporter. It is mainly found in Italy and Finland where prevalence is 1/60,000. Diagnosis requires amino acid assays in plasma and urine where increased urinary excretion and low plasma concentrations of lysine, arginine, and ornithine indicate positive diagnosis. The clinical spectrum varies from vomiting, diarrhea, failure to thrive, hepatosplenomegaly, episodes of hyperammonemic coma and mental retardation. Although clinical manifestation of LPI intolerance appeared in first two years of life, early diagnosis after the onset of symptoms, can be made.

Key Words: lysine-protein intolerance, consanguinity, Pakistan.

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P-ChemPath-0002

NEUROLOGICAL DEFICIT AT THE TIME OF PRESENTATION IN PATIENTS WITH MAPLE SYRUP URINE DISEASE: A SINGLE CENTRE POINT PREVALENCE STUDY

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ABSTRACT

Objective: Aim of this study was to determine the frequency of MSUD patients presenting with Neurological deficit at the time of presentation.

Study Design: A cross-sectional study

Material and Method: Study was performed at the biochemical genetics laboratory (BGL), AKU. Plasma Amino acid (PAA) analyzed at BGL by high performance liquid chromatography, from January 2013 to March 2018 were included. Analysis of patients reported as MSUD based on increased levels of Leucine, Isoleucine and Valine on PAA was performed. Demographic, clinical and biochemical details were extracted from BGL history form. Neurological deficit was labeled if history of hypotonia, lethargy, developmental delay, mental retardation, seizures, encephalopathy or coma was present. Data was analyzed by Microsoft Excel 2010.

Results: Total 33 MSUD patients diagnosed based on PAA were included in the final analysis. The median (Q3-Q1) age of patients was 22 days (24-10), with only 6 presenting after 1 years of age. Male to female ratio was 1.2. Parents of 73% (n=24) patients had consanguineous marriage.

Median blood pH, ammonia, lactate, SGPT and random glucose levels were 7.39 (7.47-7.3), 149ug/dl (165.45-77.7), 2.7mmol/l (12.75-1.05), 20IU/l (45.75-18) and 70mg/dl (107-46.5) respectively. While median Leucine, Isoleucine and Valine levels were 2019 mmol/L (2899-1715), 470 mmol/L (608-278) and 636 mmol/L (742-516) respectively. Neurological deficit was observed in 85% (n=28) patients, most common clinical feature was hypotonia/lethargy followed by seizures and developmental delay in 75% (n=25), 30% (n=10) and 21% (n=7) patients respectively. While encephalopathy, coma and mental retardation was observed in 24% (n=8), 18% (n=6) and 15% (n=5) patients respectively.

Conclusion: MSUD is an important cause of neurological deficit and should be looked into in patients presenting with intractable seizures, unexplained hypotonia, developmental delay, mental retardation or recurrent coma. In this context large scale awareness campaigns at both primary and tertiary care level are dire need of time.

Key Words: Maple syrup urine disease, Leucine, Isoleucine, Valine, Amino acids, Pakistan, seizures, hypotonia.

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P-ChemPath-0003

POSTGRADUATES VIEWS ON METABOLIC BONE DISEASE COURSE VIA VIRTUAL LEARNING ENVIRONMENT

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ABSTRACT

Objective: To understand the postgraduate trainees' perspective about the need of online course on Metabolic Bone Diseases (MBD)

Study Design: Descriptive, Qualitative research

Material and Methods: Focused group discussions (FGD) with PG trainees from Medicine, Radiology, Chemical Pathology and Orthopedics, were held at section of Chemical Pathology, Department of Pathology and Laboratory Medicine, AKU twice in May and July, 2018, after written informed consent. The PGs from both AKU and other institutes (connected via ZOOM) across Pakistan participated. Two faculty members moderated the discussion. The goal of FGD was to evaluate the need and challenges in participating in an online course on Metabolic Bone Diseases.

Results: The perspectives of PGs (n=14, residency years 1-6) was concluded into following themes; need of course, time commitments, tools and technology to be utilized, course content and assessment. All the PGs (14/14) agreed that there was a dire need for MBD online course. They expressed concerns that the burden of MBD was high (8/14), the objectives of MBD were not covered in their trainings (10/14) and it was important from examination point of view (11/14). The time which PGs were ready to devote to the course ranged from 15 to 20 minutes per day to 2 to 4 hours per week. PGs were comfortable in accessing the course either through cellphone or computer. All except two PGs were comfortable in using social media group as a tool for connecting with faculty and students enrolled in MBD course. PGs (10/14) responded that internet connectivity could be a hindrance, few also responded that might not be able to participate in discussions due to heavy clinical duties. Group prosecuted that live lectures require a dedicated time slot which is difficult to manage with routine duties and majority were in favor of downloadable short presentations. They had a consensus regarding the use of MCQs as the mode of assessment. They accentuated that the focus of MCQs should be on diagnostic approach and interpretation of investigative work up. Two participants emphasized that a final assessment should be planned at the end of the course in order to qualify.

Conclusion: It was clear that there is a need for a comprehensive MBD course. To make it successful the challenges PGs identified should be catered when the course is developed. Course can be split into multiple modules, course content should be clinical, not too time demanding, lectures and simulated clinical cases can be video recorded. Feedbacks, reading material and questions can be shared using social media. The detailed information about PG's perceptions and opinions will help design a successful MBD online course tailored according to their needs.

ERC number: 5415-Pat-ERC-18

Key words: Metabolic Bone Diseases, Postgraduate trainees, Virtual learning.

P-ChemPath-0004

URINARY SUCCINYLACETONE: A DIAGNOSTIC MARKER FOR TYROSINEMIA TYPE-1

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ABSTRACT

Objective: To determine the frequency and clinical spectrum of Tyrosinemia type 1 in patients tested for urine Succinylacetone by gas chromatography-mass spectrometry (GCMS).

Study Design: An observational study.

Methods: **S**tudy was conducted at the Biochemical Genetic Lab (BGL), section of Chemical Pathology, Department of Pathology and Laboratory Medicine. Patients tested for urinary succinylacetone from January 2015 to October 2017 at the Biochemical Genetic Lab, AKU were included in this study. The urine samples were quantified for succinylacetone levels by GCMS. Clinical and biochemical data was collected from the structured BGL requisition forms. Data was analyzed by Microsoft Excel 2010.

Results: A total of 140 patients were tested for urine succinylacetone and 17 (12%) showed elevated succinylacetone levels. Median age was 450 days (715-202) with 9 (53%) male patients. The median urinary succinylacetone level in patients with Tyrosinemia type I was 187mmol/mol Cr (419-114), while mean ±SD AFP, SGPT levels were 8000± 696 IU/mL, and 65 ± 58.7 IU/L respectively. In patients with Tyrosinemia type-1, parents of 14 (82.3%) had consanguineous marriage. The most common symptom was failure to thrive 10 (58.8%) followed by jaundice 8 (47.0%) and fever 5 (29.4%). Hepatomegaly was found in 11 (64.7%) of the patients while urine smelt like rotten vegetables in 4 (23.5%) of patients. However, data of liver biopsy, ultrasound, C.T scan and MRI were not available.

Conclusion: Succinylacetone is a diagnostic biochemical marker for hereditary Tyrosinemia type-1 and more than one tenth of patients suspected with Tyrosinemia type-1 showed high urine succinylacetone.

Keywords: Tyrosinemia, Succinylacetone, Organic acids, Pakistan, Gas chromatography-mass spectrometry.

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P-ChemPath-0005

COMPOSITION OF STONES AND METABOLIC RISK FACTORS IN INFANTS. AN ENDEMIC COUNTRY EXPERIENCE

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ABSTRACT

Objective: The aim of this study was to investigate socio-economic factors, history, chemical composition and urinary risk factors in infants upto 2 years to develop preventive and appropriate metaphylaxis for recurrence of the disease.

Study Design: Cross-sectional retrospective study.

Material and Methods: Between 1982-2016, 1437 infants presented to our institute with 1217 stones. Patients were evaluated for demographics, blood and 24 h urine for calcium, magnesium, phosphate, uric acid, electrolytes and additional protein, citrate, ammonia and oxalate in urine. Chemical composition of stones was analyzed by Fourier transformation infrared spectroscopy (FTIR). All reported values were two sided and statistical significance was considered at *p* value ≤0.05.

Results: The mean age of infants was 17.5±6.24 months with a M:F ratio of 5:1. Nearly half (50%) of the infants were rural dwellers, 92% belonged to low socio-economic class and 70% were malnourished. A history of chronic diarrhoea was reported in 17% and urinary tract infections in 8%. Overall frequency of compounds in stones showed Ammonium acid urate (AAU) in 75%, Calcium Oxalate (CaOx) in 40%. Uric Acid (UA) in 12%, Calcium phosphate apatite (CaP) in 7%, Magnesium ammonium phosphate (Struvite) in 5%), Cystine in 1% and Xanthine in 5%. Urinary metabolic abnormalities showed Hypocitraturia in 84%, hyperoxaluria in 26%, hyperuricosuria in 52%, hyperammonuria in 14%, hyponatriuria in 49% and hypovolemia in 50%.

Conclusion: Our study has shown that AAU is a major component of stones in infants where the main risk factors are poverty, malnutrition, diarrheal diseases and dehydration.

Key Word: Chemical composition, Urinary Stones, Infants, metabolic, risk factors

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P-ChemPath-0006

ROLE OF EQAS IN ORDER TO SHOW THE PERFORMANCE OF THE CLINICAL LABORATORY

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ABSTRACT

Objective: The goal of this study is to review EQAS results over a time period of one year in an order to show the performance of our laboratory.

Study Design: This cross-sectional study was done from June 2017 July 2018 Medicare Cardiac and General hospital, Jinnah medical and dental college, Karachi

Material and Methods: Twelve lyophilized EQAS samples were received at department of clinical biochemistry. On a quarterly basis that needed to be stored, reconstituted and analyzed as per the guidelines and schedule provided by the organizing EQAS body. For each month unknown / blind sample provided by the EQAS body, reconstituted on scheduled dates and analyzed for the parameters for which our laboratory participated. The results were uploaded on the EQAS website on the schedule dates and our performance score was downloaded after completion of each month.

Results: The study revealed very good scores with 97%,98% and 100%;98%,94%:and 96% and 96, 94% and 99% of the total results falling in the very good performance score category in the three quarters of year between June 2017-Sep 2017, October 2017-January 2018.February 2018-May2018 respectively in terms of Variation Index. Score (VIS). This study also revealed discrepancies in the performance of a few parameters especially phosphorus (Phos), bicarbonate (HCO3), potassium(K) and sodium (Na) in few occasions of this cycle.

Conclusion: This study revealed that errors in our performance guide us to take corrective actions and thus to improve the quality of our laboratory reports. Good performances provide confidence that patients are providing proper diagnosis and management esp. critically care services where accurate diagnosis is most urgent required

Results: Participation in EQAS over the last year has helped us significantly to improve our laboratory services.

Key Words: External quality assessment scheme, Laboratory, Variation index score (VIS)

P-ChemPath-0007

BRIDGING THE GAP BETWEEN TRAINEES & SUPERVISORS: SUPPORTING THE NATIONAL RESIDENCY PROGRAM IN CHEMICAL PATHOLOGY IN PAKISTAN USING A VIRTUAL PLATFORM

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ABSTRACT

Objective: College of Physicians and Surgeons Pakistan (CPSP) runs a comprehensive four years structured residency program in Chemical Pathology with accredited centers across the country. Trainees often carry unanswered questions and misperceptions regarding certain areas in this sub specialty training which can be more expansively resolved by a faculty sitting at a different center. Our objective was to virtually connect Chemical Pathology trainees from across Pakistan with faculty quantify the feedback gathered from participants.

Study Design: Descriptive cross-sectional study

Material and Methods: An interactive zoom session using was organized by the section of Chemical Pathology, Department of Pathology and Laboratory Medicine, AKU Karachi Pakistan in collaboration with Pakistan Society of Chemical Pathology (PSCP) on 26th April 2018. A pre-structured program was developed based on talks by various supervisors across the country and discussion sessions. The program and instructions to connect via ZOOM was circulated via e-mail and Whatsapp to ensure maximum participation of trainees and faculty. Feedback from participants gathered at AKU (n=17) was analyzed using Likert's scale of 1-5 (poor, average, good, very good, excellent). Strengths, weakness and suggestions were also recorded.

Results: Participants from 17 centers across Pakistan including 8 from Karachi, 3 from Rawalpindi, 2 from Lahore and 1 from Bahawalpur, Rahim Yar Khan, Peshawar and Quetta respectively connected via ZOOM. A total of 68 participants attended the session including 27 faculty members. There were 5 focused areas of discussion including overview of curriculum and assessment in IMM, update of expected changes in exam, tips for trainees to ace the residency, opportunities in upcoming cities of Pakistan and way forward. Majority of the queries (n=7) were regarding topics for the IMM exam followed by explanation of marking in practical (n=3). On feedback analysis objectives of the study were defined yielded a score of 5(82%). 100 % agreed that presentations were at the level of participants understanding. 71% gave a score of 5 to interaction and content coverage. 82% graded responses to queries and organization as excellent. The overall assessment of the activity was rated as 5 by majority of the participants (82%).

Conclusion: The detailed interactive session helped resolve trainees' perceptions and queries which will enable them to prepare for the assessments more extensively.

Keywords: Curriculum, Exam, Virtual.

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P-ChemPath-0008

DIAGNOSTIC VALIDATION OF FULLY AUTOMATED ANA DETECTION BY INDIRECT IMMUNOFLUORESCENCE: INITIATIVE TOWARDS STANDARDIZATION

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ABSTRACT

Objective: To determine the diagnostic validation of novel automated system for ANA detection by indirect immunofluorescence in comparison to manual method.

Material and Methods: The observational study was conducted in Department of Chemical Pathology and Immunology, Chughtai Institute of Pathology for a period of three months from October 2017 till December 2017. The ANA-IIF testing of total 130 serum samples were performed by manual assay using slides coated with HEp-2 cells. Results of manual assay were obtained by visual interpretation of the fluorescence patterns observed under a LED fluorescence microscope. In parallel, all 130 samples were also subjected to a fully automated ANA-IIF assay for initial slides processing, microscopy and image capturing and display of patients specific ANA patterns. The serum samples having titers more than 1:80 were considered ANA positive either by visual or automated method and further dilutions (1: 160, 1: 320, 1: 640, 1: 1280) were made in a stepwise manner (2-3 dilutions for each step) until either a negative result or a positive result for the highest dilution was obtained. Positive ANA-IIF results were categorized by the common predominant ANA pattern in the highest dilution with a positive result as being either homogeneous, speckled, nucleolar, centromere, mitochondrial, or nuclear dots.

Results: A total of 130 cases are analyzed by manual and automated ANA IIF each. For diagnostic validation method comparison was done and passing the automated method for patient testing. The concordance between two methods showed over all agreement of 95.9% with positive agreement of 97.7% and negative agreement of 95.0%. Cohen kappa value is 91.2% (83.7 to 98.8%) which showed strong correlation between the two methods. Automated IIF method showed diagnostic sensitivity at CI 95 % is 89.58 % (77.34 % to 96.53 %) and diagnostic specificity at CI 95 % is 95.12 % (87.98% to 98.66%). The breakdown of pattern recognition by both methods are summarized in Table 1 suggesting commonest pattern being homogenous for which confirmatory test is DsDNA.

Conclusion: A strong correlation exist between automated and manual ANA detection methods by IIF. This novel fully automated ANA by IIF was introduced for patient testing in our laboratory which offers the advantage of faster and much easier performance as well as better harmonization in the interpretation of the patients' results.

Key Words: Antinuclear antibody, Indirect immunofluorescence, Automation

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ChemPath-0009

FREQUENCY OF SUBCLINICAL HYPOTHYROIDISM IN A COHORT OF PEOPLE ATTENDING THE ENDOCRINE LABORATORY OF COMBINED MILITARY HOSPITAL, QUETTA

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ABSTRACT

Objective: To determine the frequency of subclinical hypothyroidism (SCH) in a cohort of people attending the endocrine laboratory of Combined Military Hospital (CMH) Quetta and find its association with gender and age.

Study Design: Descriptive cross-sectional

Place and Duration of Study: CMH Quetta from January2017 to June 2017.

Material and Methods: Through consecutive sampling, 532 individuals, reporting for thyroid function evaluation were included. Twenty subjects with a history of medication for a thyroid disorder, thyroidectomy, or exposure to radioiodine were excluded. Venous samples for thyroid stimulating hormone (TSH), total T3 (TT3), and free T4 (FT4) were analyzed using Siemens kit (chemiluminescent method). The statistics were done with SPSS version 20. Chi-square analysis and binary logistic regression were used to evaluate association of age and gender with SCH.

Results: Of 512 included patients (mean age: 33.6 ± 13.9 years), majority (56.4%) were female. The mean values for TSH, FT4, and TT3 were 6.19 ± 14.67 mU/L, 15.57 ± 7.43 pmol/l, and 1.45 ± 0.39 nmol/l respectively. The frequency of SCH was 75 (14.6%), while for overt hypothyroidism, it was 24 (4.7%). SCH was significantly more common in females ($p<0.001$) but was not associated with age.

Conclusion: The prevalence of SCH in our sample was high, comparable to earlier Pakistani data. The female gender was significantly associated with SCH.

Key Words: Subclinical hypothyroidism, Frequency, Quetta.

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P-ChemPath-0010

ASSOCIATION OF SERUM SEX HORMONE BINDING GLOBULIN WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Objective: To investigate the association of circulating levels sex hormone binding the globulin with type 2 diabetes mellitus.

Study Design: Case control / analytical study

Place and Duration of Study: Department of Chemical Pathology Army Medical College, Rawalpindi/ Department of Endocrinology Military Hospital, Rawalpindi [Nov 2014 to Nov 2015].

Results: In this low level of SHBG (39±22.25 vs 62.35±32.52, P=<0.05) were noted in diabetic as compared to the control participants. The diabetic patients presented with significantly higher fasting plasma glucose (11.23±3.65 vs. 4.35±0.68, P=<0.05), HbA1c (6.84±0.48 vs. 5.31±0.48, P=<0.05), serum insulin (8.90±6.51 vs. 6.32±4.09, P=<0.05) and insulin resistance (4.84±5.18 vs. 1.23±0.83, P=<0.05), calculated by HOMA IR. SHBG was negatively associated with HbA1c (r-101), FPG (r-107), serum insulin (r-132) and insulin resistance, IR (r-142) in the diabetic group.

Discussion: Serum SHBG levels were significantly lower in the diabetic ($p<0.01$) as compared to the control group. Same results are reported by Ding *et al* (2009), Goto *et al* (2012), Lakhman *et al* (2010), Vikant *et al*. (2010), Stellato *et al* (2000).

Conclusion: This study confirmed the association of decreased SHBG levels with type 2 diabetes mellitus. Moreover, these decreased levels were also associated with poorly controlled glycemic status in the diabetic patients. Therefore, it is concluded that SHBG has a potential to be used a biomarker of metabolic control in type 2 diabetes mellitus.

MICROBIOLOGY SCIENTIFIC SESSION ABSTRACTS

SS-Micro-0001

ARE WE HEADING TOWARDS POST ANTIBIOTIC ERA- CLINICIANS NIGHTMARE FROM A MICROBIOLOGIST'S VIEW POINT

Brig Irfan Ali Mirza
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ABSTRACT

Since the discovery of Penicillin by Alexander Fleming, Antibiotics have served the mankind for the last about eight decades. Clinicians have relished and relied heavily on multitude of antibiotics to treat life threatening infections during all these times. No sooner the clinicians felt comfortable, during the last five decades health care facilities around the globe have experienced the emergence of microorganisms like Methicillin resistant staphylococcus aureus (MRSA), Vancomycin resistant enterococcus (VRE), Extended spectrum beta lactamases (ESBL), Carbapenem resistant Enterobacteriacae (CRE), Carbapenem resistant Acinetobacter baumanni (CRAB) and many more antibiotic resistant microorganisms that microbiologists and clinicians have to memorize the alphabetical acronyms and abbreviations. The emergence of XDR-TB and XDR-Salmonellae have added fuel to the fire in resource limited countries like Pakistan.

With the threat of post antibiotic era haunting the health care providers, it has become mandatory to come up with national action plan. The **way forward** lies in finding the tangible and practical steps to control this tide. The two most important players in the quest for control are the **clinicians** and **microbiologists** whose nexus can play a very vital role against this battle

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SS-Micro-0002

NEXT GEN SEQUENCING

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ABSTRACT

Typhoid is endemic in Pakistan with a high mortality. Resistance to third-generation cephalosporins and fluoroquinolone antibiotics is being reported by various hospitals in the country. Sensitivity to 4th generation cephalosporins is usually not reported in our laboratories, hence resistance to this antibiotic is not known**.** A recently reported 3rd generation cephalosporin and quinolone resistant Salmonella enterica serovar Typhi isolated from a patient suffering from typhoid at Military Hospital, Rawalpindi was, however, found to be resistant to cefepime (4th generation cephalosporin) as well, on further testing. As it was the first known case of Salmonella Typhi showing resistance to a fourth-generation cephalosporin, it was sent to the Centre for Genome Sciences and Systems Biology, Washington University School of Medicine, Missouri, USA for molecular typing in collaboration with NUST (National University of Science and Technology). Whole Genome sequencing was carried out to identify the resistance determinants carried by this *S* Typhi isolate. Genomic DNA from 10 colonies of the isolate was extracted with the bacteremia DNA kit (Mo Bio) and used as input for sequencing libraries using a modification of the Nextera XT kit (Illumina). A total of 10,307,350 paired-end 150-bp reads were generated from an Illumina NextSeq 2500 and processed using the High-Throughput Computing Facility at the University Medical School. The potentially contaminating human DNA was removed and 4,975 coding sequences (CDSs), 69 tRNA genes, and 4 rRNA genes were identified. The resistance to cefepime and fluoroquinolnes (cipro-floxacin, levofloxacin, and moxifloxacin) was explained by identification of the genes, using the databases, as *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-1}}$, S83F *gyrA*, and *qnrS1*. The prevalence of *bla*$_{\text{CTX-M-15}}$-positive *S*. Typhi isolates has been reported in other countries like Iraq, Kuwait, India, and Bangladesh (14–17). This study was the first draft genome sequence of a *bla*$_{\text{CTX-M-15}}^{-}$, *bla*$_{\text{TEM-1}}^{-}$, and *qnrS*- positive *S*. Typhi strain from Pakistan exhibiting resistance to cefepime and fluoro- quinolones.

(Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number NIFP00000000.

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SS-Micro-0003

MICROBIOLOGY QUALITY CONTROL SAMPLES – THE NEQAPP EXPERIENCE

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ABSTRACT

National External Quality Assurance Programme Pakistan (NEQAPP) is nonprofit activity being run by AFIP to contribute towards the assurance of quality in laboratories at national level in the interest of public health and safety. It

was started in 1996 specifically for chemical pathology & later on expanded to all disciplines of pathology including Microbiology. 45 Laboratories, both civilian and military are registered in the microbiology programme. In the beginning of every quarter of the year, samples are delivered out to these labs for culture, identification of isolate and its susceptibility pattern. Results are discussed for timely submission, identification and incorrect susceptibility issues. Data reflects the last two years of experience incorporating the observations related to inappropriate susceptibility testing methods.

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SS-Micro-0004

ANTIBIOTIC STEWARDSHIP: A TEAM APPROACH

Dr Mateen Izhar
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ABSTRACT

This presentation will review a set of coordinated strategies to improve the use of antimicrobial medications with the goal of enhancing patient health outcomes, reducing resistance to antibiotics, and decreasing unnecessary costs.

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SS-Micro-0005

EMERGENCE OF MALEIDOSIS IN PAKISTAN

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ABSTRACT

Melioidosis is an infection caused by the intracellular gram-negative bacterium, *Burkholderia pseudomallei.* It occurs predominantly in Southeast Asia, northern Australia, South Asia (including India), and China. The agent is listed as Category B bioterrorism agent by CDC, USA. The infection has never been reported among humans in Pakistan.

Case report: A 75-year-old diabetic male, was brought to ER of Shifa International Hospital, Islamabad, in January 2018 with history of fever and severe leg wound infection. His blood complete picture showed WBC count of 14700 / ul (Neutrophils 70%) and Haemoglobin 9.4 g/dl, CRP was 176 mg / L. Blood and wound swab culture revealed Non-lactose fermenting, oxidase positive growth. It was initially identified as *Pseudomonas* species. The organism could not be identified with API 20NE, but Vitek 2 identified the growth as *Burkholderia pseudomallei.* On disk diffusion susceptibility testing the isolate appeared susceptible to ceftazidime and amoxicillin-clavulanate, but resistant to gentamicin. The isolate was sent to CDC USA through NIH Islamabad for confirmation. A positive *Burkholderia pseudomallei* PCR was reported by CDC, USA. **Conclusion:** Emergence of melidiosis in Pakistan is a significant new public health concern. Diagnosis of the infection can be challenging. Microbiology labs should keep in mind that any *Pseudomonas* species susceptible to ceftazidime and amoxicillin-clavulanate, but resistant to gentamicin and/or identified as *Burkholderia pseudomallei* by API 20NE or Vitek 2 needs confirmation with a PCR test.

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SS-Micro-0006

ENTERIC FEVER- CHALLENGES IN THE DIAGNOSIS AND CONTAINMENT OF XDR *SALMONELLA* TYPHI

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ABSTRACT

Enteric fever caused by Salmonella Typhi and Paratyphi, is a potentially life-threatening illness that is typically transmitted through contaminated water and food. Blood culture remains the mainstay for the laboratory diagnosis. However, diagnosis remains a challenge especially in second and third week of illness. Serodiagnosis depends upon the appearance of antibodies in the blood. Available modalities lack sensitivity and specificity. Molecular tests are promising, but are not widely available in endemic regions. Due to the ongoing outbreak of enteric fever due to XDR Salmonella Typhi in Pakistan, CDC has issued a level 2 travel alert for this country. Therefore, it is essential for health officials; to take measures to stop the circulation of this notorious pathogen in the community by implementing mass scale vaccination programs on immediate basis, improve quality of drinking water and sanitation all over the country. These strategies will assist in the ultimate reduction of excessive use of precious antimicrobials, another area of concern in the world of infectious diseases.

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SS-Micro-0007

CURRENT INNOVATIONS IN LABORATORY DIAGNOSIS OF LIFE-THREATENING INFECTIONS

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ABSTRACT

Approximately 70% of the medical decisions for the management of patients are taken on the basis of laboratory results. Several key results such as biochemistry and hematology can be provided within the day of entrance into the hospital. There is a need to accelerate the microbiologic diagnostic techniques. This is particularly true in the case of life-threatening infections like sepsis, meningitis etc, for which appropriate decisions and treatments should be provided in a critical time window of 6 hours to reduce morbidity and mortality. Similarly, diagnosis of organisms that are either slow or difficult to culture is another problematic area of diagnostic microbiology.

The evolution of molecular biology and improved serological methods in the last decade provide tools to detect etiological agents of infections that would otherwise remain undetected. In the present talk, I will discuss the advantages and limitations of these new molecular and serological approaches that at best complement the culture-based diagnosis of some life-threatening infection.

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SS-Micro-0008

EMERGING TECHNOLOGIES FOR MOLECULAR DIAGNOSIS OF SEPSIS

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ABSTRACT

Sepsis is a serious and life-threatening clinical condition that generally results from a primary bacterial infection or less frequently, from a fungal and/or viral infection. Affecting nearly 1 out of every 23 hospitalized patients, it is the sixth most common reason for hospitalization.

Rapid and accurate diagnosis of sepsis remains a significant challenge in modern health care. Despite achievements in molecular diagnostic techniques, blood culture analysis remains the gold standard for diagnosing sepsis. However, this method is too slow to significantly influence the treatment of patients. The rapid initiation of precise and targeted antibiotic therapies depends on the ability of a sepsis diagnostic test to identify organisms along with antimicrobial resistance within 1 to 3 h. It should utilize small sample volumes and detect polymicrobial infections and contaminants.

I will try to outline the limitations of routine blood culture testing and discuss how emerging sepsis technologies are converging on the characteristics of the ideal sepsis diagnostic test. The ideal technology should include the following characteristics:

1. Rapid detection of the pathogen in less than 3 h
2. Broad-based detection, including bacteria, viruses, and fungi
3. Minimal invasiveness, utilizing clinical samples with low specimen volumes
4. High sensitivity and specificity for the immediate initiation of targeted antibiotic use in the presence of signs and symptoms of systemic inflammation
5. Polymicrobial detection of pathogens in the presence of contaminants across a wide range of pathogen loads
6. Integration into the clinical work flow
7. The ability to detect unknown and emerging pathogens
8. The ability to distinguish the inflammatory response as being either host or pathogen driven

I will discuss seven molecular technologies that have been validated on clinical blood specimens or mock samples using human blood. These include IridicaPlex ID, Septifast, Septitest, Nanopore sequencing (MinION),U-dHRM and machine learning on pathogen DNA fingerprints.

An exciting new era of molecular diagnostics for bloodstream infections is emerging through innovations in sample preparation, single-molecule detection methods, sequencing, and applications of machine learning. However, each emerging technology harbors unique benefits and drawbacks. These emerging technologies have the potential to identify microorganisms and provide relevant subspecies and antibiotic resistance information in a clinically relevant time frame that is much shorter than that currently required for blood culture. Such an integrated approach may overcome the limitations of each technology individually to facilitate targeted and precise antibiotic use.

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MICROBIOLOGY FREE PAPER ABSTRACTS

FP-Micro -0001

ANALYZING THE ACCURACY OF LOOP MEDIATED ISOTHERMAL AMPLIFICATION IN THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS IN PAKISTAN

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ABSTRACT

Objective: The study was done to determine the adequacy of Loop-mediated Isothermal Amplification for the detection of *Mycobacterium tuberculosis*.

Design: A cross sectional study was done on 72 clinical sputum samples taken from the patient suspected of tuberculosis using a novel technique LAMP targeting IS6110 gene sequence for the first time in Pakistan.

Material & Methods: Six primers recognizing eight distinct regions on the target sequence were employed. The assay was performed in total volume of 25 µl containing primers, DNA polymerase, Fluorescent dye and sample incubated at 65°C for 60 minutes along with positive and negative controls. LAMP amplicons were detected by their fluorescence under UV light and compared with gold standard MGIT culture system and smear microscopy.

Results: Out of 72 total samples 60(83.3%) were LAMP positive and 61(84.7%) were MGIT positive. Its specificity, sensitivity is 100% and 98.36% respectively with PPV 100% and NPV 91.6%.

Conclusion: According to our study the LAMP assay is suggested to be a potential nucleic acid based diagnostic method for TB detection in developing countries.

Key Words: Loop Mediated Isothermal Amplification (LAMP), IS6110 gene, *Mycobacterium tuberculosis*. Mycobacterium Growth Indicator Tube (MGIT).

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FP-Micro-0002

MOLECULAR SURVEILLANCE OF DRUG RESISTANCE: PLASMODIUM FALCIPARUM ARTEMISININ RESISTANCE SNPS IN KELCH PROTEIN PROPELLER DOMAIN FROM SOUTHERN PAKISTAN

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ABSTRACT

Objective: We describe genotyping of point mutations in Kelch protein propeller domain of Plasmodium falciparum associated with artemisinin resistance

Study design: Prospective observational Study

Material and Methods: Samples (N=116) were collected from patients with microscopy confirmed *P. falciparum* malaria attending Aga Khan University Hospital during September 2015-December 2017.DNA was isolated using the whole blood protocol for the QIAmp DNA Blood Kit. The K13-propeller gene (K13) was amplified using nested PCR. Double-strand sequencing of PCR products was performed using Sanger sequencing methodology. Sequences were analyzed with MEGA 6 and Bio-edit software to identify specific SNP combinations.

Results: All isolates analyzed for K13-propeller allele were observed as wild-type in samples collected post implementation of ACT in Pakistan. C580Y, A675V, Y493H and R539T variants associated with reduced susceptibility to ACT were not found. K189T polymorphism was found in 2 isolates not significantly associated with Artemisinin resistance.

Conclusion: K13-propeller polymorphism are useful molecular marker for tracking the emergence and spread of ART-resistance in P. falciparum. C580Y polymorphism is reported from Cambodia and Ghana with rapid invasion of the population and almost near fixation in south East Asia. Surveillance of K13 polymorphism is necessary as recommended partner drug for Pakistan sulfadoxine–pyrimethamine (SP) has shown reduces susceptibility which will compromise the efficacy of fast acting ACT. Surveillance of anti-malarial drug resistance to detect its emergence and spread need to be strengthened in Pakistan.

Key Words: Plasmodium falciparum, Artemisinin resistance, Kelch protein propeller domain

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FP-Micro -0003

CANDIDA AURIS; AN EMERGING SUPERBUG IN OUR HOSPITAL SETTINGS

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ABSTRACT

Objective: *Candida auris* is an emerging fungal superbug around the globe due to its nosocomial transmission. It can cause hospital acquired infections with poor outcome mainly in critically ill patients. The bug is gaining popularity as it is difficult to diagnose, treat and eradicate. We report on going rise of *Candida auris* infections in our intensive care units causing candidemia during June 2018 to October 2018. We describe here the outbreak identification, investigations and implementation of infection control measures to prevent its further spread.

Materials & Methods: Data on *C. auris* case demographics, environmental screening, implementation of infection prevention/control measures, and antifungal susceptibility of patient isolates were prospectively

recorded and analyzed. We also analyzed our previous one year data retrospectively to see whether we had similar such cases. All the clinical samples received from intensive care units were dealt as per standard protocol. Speciation of *C. auris* was performed by VITEK 2 system version 08.01.

Results: This report describes an ongoing outbreak of 14 *C. auris* cases within a 45 beded intensive care units. A total of 31% (n = 14/ 45) patients developed proven *C. auris* infection with a candidaemia rate of 20% (n = 9/45). Environmental and patient sampling showed persistent presence of the yeast around bed space areas, nasal, web, CVP tip and NG tubes. Implementation of strict infection and prevention control measures included: isolation of cases and their contacts, wearing of personal protective clothing by health care workers, screening of patients on affected wards, skin decontamination with 2% chlorhexidine, environmental cleaning with chorine-based reagents and hydrogen peroxide vapour. Phenotypic methods demonstrated that *C. auris* isolates from the same geographic region clustered, but single source could not be substantiated in our study. Previous one-year data analysis revealed only two sporadic cases of *C. haemulonii* but clinical isolates were not available for confirmation / further workup.

Conclusion: For early diagnosis, high level of suspicion should be made when isolating a non-albican *candida* spp. with fluconazole resistance. Prompt treatment only in invasive candidiasis is necessary due to limited treatment options. Strict adherence to infection control measures can prevent the further spread of this deadly organism in future**.**

Key Words: *Candida auris,* Outbreak, Hospital acquired infections, Nosocomial transmission.

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FP-Micro-0004

EMERGING THREATENING ANTIBIOTIC RESISTANCE IN *ESCHERICHIA COLI*-A DIAGNOSTIC LABORATORY EXPERIENCE

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ABSTRACT

Objective: *Escherichia coli* is among the most commonly isolated pathogens from clinical specimens. However, during our routine lab work it is alarmingly seen resistant to multiple classes of antimicrobials. Keeping in view this observation, we conducted this study to determine the current trends of antimicrobial resistance and possible empirical therapeutic options in clinical isolates of *E.coli* from various clinical samples.

Material and Methods: It was a descriptive, cross-sectional study. Data of all the cultures that yielded *E.coli* was collected from the database of the institute, Laboratory Information Management System (LIMS), for past 1 year; from July 2017 to June 2018. Antibiotic susceptibility testing was performed using modified Kirby Bauer disc diffusion method as per latest CLSI guidelines. For reporting colistin resistance MICs were performed by Vitek 2 systems-version 08.01.

Results*:* A total of 2249 *E.coli* were isolated during the study period, from 648 (28.8%) indoor and 1601 (71.2%) outdoor clinical samples. Overall, ESBL producers were 75.4%, Carbapenem resistant isolates were 13.4% and colistin resistant isolates were 1%. The proportion of ESBL producers and carbapenem resistant isolates were significantly higher in indoor cases (ESBL 86.3%, CRE 22.1%) than outdoor cases (ESBL 70.9%, CRE 9.9%). The antimicrobial resistance trends were found to be rising in the latter half of the year (Jan-Jun 2018) than in the earlier half (Jul-Dec 2017) in both indoor and outdoor cases. Most resistant isolates were recovered from the respiratory specimens (ESBL 89%, CRE 39.1%) followed by blood, body fluids and infected wounds. Urine showed the least resistant isolates (ESBL 68.3%, CRE 6.2%). Overall 9 isolates (1%) were found colistin resistant.

Conclusion*:* Increasing antibiotic resistance in *E.coli* has turned it into an emerging superbug in our setup. More than three fourths of our isolates are ESBL producers which reaches around 89% in respiratory specimens, where around 39% *E.coli* are carbapenem resistant as well. Now, colistin resistant isolates are also being isolated. This is seriously an alarming scenario where treatment of simple bacterial infections is becoming a hard task.

Key Words: *Escherichia coli*, ESBL, Antimicrobial resistance, Carbapenem resistance, Colistin resistance.

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FP-Micro -0005

ANTIMICROBIAL RESISTANCE AND CONSUMPTION IN THE CRITICALLY ILL POPULATION; FACING THE CHALLENGE IN TERTIARY CARE CENTER OF A LOWER MIDDLE INCOME COUNTRY

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ABSTRACT

Objective: This study was conducted to report the antimicrobial resistance patterns in ICU settings, to highlight the prevalence of resistant isolates in the area and to evaluate the antimicrobial consumption by the critically ill population of our setup.

Study Design: Descriptive cross-sectional study, conducted in the department of microbiology, Combined Military Hospital, Lahore from January 2017 to December 2017.

Material & Methods: All patients admitted to the ICUs for the study period and who received antibiotic treatment were included from admission until either discharge, transfer, or expiry. Sputum, endotracheal aspirates, BAL, blood, urine, stool, catheters, fluids including CSF and pus samples were collected, based on clinical indications of infections during patients' ICU stays. All bacteria isolated

were included in the study with exclusion of repeat isolates. The samples were dealt in the laboratory using standard microbiological procedures as per guidelines of American Society of Microbiology (ASM) in clinical microbiology procedures handbook. The isolates were identified using respective API galleries and antibiotic susceptibility testing was performed by Kirby-bauer disk diffusion method using breakpoint definitions of Clinical and Laboratory Standards Institute (CLSI).

The antimicrobial usage was evaluated for the main ICU which is a 12-bed adult ICU. The data on antibiotic consumption was collected for the study year's last quarter (October to december2017). For each antimicrobial Antibiotic consumption was expressed as defined daily doses (DDD) per 100 occupied bed days (DDD100).

Results: The susceptibility profiles of enterobactericeae, *Pseudomonas, Acenetobacter baumanii* and *Staphyl-ococcus* species revealed high rates of resistance among commonly used antimicrobials in ICUs. Resistant phenotypes like carbapenem resistant enterobacteriaceae (41.5%), MDR *pseudomonas* (63.8%) and carbapenem resistant *Acenetobacter baumanii* (100%) were prevalent. Antimicrobial consumption revealed a striking high usage of colistin (27.87 DDD/100 patient days) followed by meropenem and moxifloxacin.

Conclusion: AMR is a complex global public health challenge, and no single or simple strategy can fully contain the emergence and spread of antimicrobial resistant organisms. Deescalation of empiric antibiotics based upon culture results and antimicrobial consumption studies is of essence in the ICU settings.

Key Words: Antimicrobial consumption, Intensive care unit, antimicrobial resistance, critically ill patients.

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FP-Micro -0006

DETECTION OF *H. PYLORI* AND *MLH1* GENE EXPRESSION ANALYSIS BY PCR IN PATIENTS OF CHRONIC GASTRITIS

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ABSTRACT

Objectives: To compare the results for detection of *H. pylori* by rapid urease test, Giemsa stain and PCR. To determine relative gene expression of *MLH1* in patients of chronic gastritis infected with *H. pylori* as compared to patients of chronic gastritis without *H. pylori.*

Study design: Descriptive study

Material & Methods: Three gastric biopsies were collected from n=20 patient undergoing upper GIT endoscopy. One gastric biopsy specimen was used for the rapid urease test. Second along with the first one i.e. rapid urease test tissue gastric biopsy specimens was kept in 10% formalin for fixation. H & E staining and Giemsa staining was performed. Third gastric biopsy specimen was urgently kept and transported in liquid nitrogen and stored at -80°C for *H. pylori* and *MLH1* gene detection by PCR

Results:

Rapid urease test: Group A comprised of (n=34) patients of chronic gastritis infected with *H. pylori* and positive on rapid urease test. Group B comprised of (n=15) patients of chronic gastritis infected without *H. pylori* and negative on rapid urease test. Rapid urease test sensitivity and specificity was100% by taking Giemsa stain as gold standard and rapid urease test sensitivity was 91.89% and specificity 100% by taking PCR as gold standard by applying Wilson score.

Histopathological examination: Chronic gastritis were observed in all forty-nine (n=49) biopsies.

Giemsa staining: H. *pylori* was observed by Giemsa staining in all thirty-four n=34 (69.4%) biopsies of group A and negative for H. *pylori* in all fifteen n=15 (31.6) biopsies of group B. Giemsa stain sensitivity (91.89%) and specificity (100%) by taking PCR as gold standard. The sensitivity and specificity both tests rapid urease test and Giemsa staining showed equal results against PCR.

***H. pylori* detection by PCR:** PCR amplified and identified *H. pylori* in all thirty-four n=34 (69.4%) biopsies of group A and from group B three n=3 (6.1%) were amplified and detected and twelve n=12 (24.5%) were not.

***MLH1* gene Expression Analysis:** *MLH1* gene was expressed on all gastric biopsies but its expression was less in chronic gastritis with *H. pylori* patients as compared to the patients of chronic gastritis without *H. pylori*.

Conclusion: As compared to histochemical stains PCR is most sensitive and specific, superior and most authentic test for the detection *H. pylori.* Rapid urease test should be performed for the urgent and rapid diagnosis of *H. pylori.* While PCR should be performed as a confirmatory test on cases of chronic gastritis negative for *H. pylori* on Giemsa staining. *MLH1* gene expression analysis was expressed in cases of chronic gastritis but its expression was less in group A (patients of chronic gastritis infected with *H. pylori* and positive on rapid urease test) as compared to group B (patients of chronic gastritis infected without *H. pylori* and negative on rapid urease test). This needs further exploration by carrying out further studies on larger sample size.

Key Words: Hematoxylin& Eosin, *Helicobacter pylori, MutL homolog,* Polymerase chain reaction.

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FP-Micro -0007

POLYMYXIN RESISTANCE IN GRAM NEGATIVE ORGANISMS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: Resistance to antibiotics is of significant concern to the health of the general population. Gram-negative bacteria (GNB) resistance is especially concerning to microbiologist and clinicians due to their rapid spread

and limited treatment options. Rapidly evolving antibiotic resistance to *Enterobacteriaceae* poses threat to existing antibiotics.

Study design: Cross sectional

Material & Methods: Colistin susceptibility was performed as follows: broth microdilution using 96-well microtiter plates to perform MICs using cation adjusted Mueller Hinton broth as culture medium. Drug concentration ranging from 0.05 to 16 µg/mL was used. Colistin sulfate powder was obtained from Sigma-Aldrich Co. (St Louis, MO, USA). ATCC 25922 *E. coli* and ATCC 27853 *P. aeruginosa* were used as quality control strains. Results were read at 24 hours and interpreted using colistin cut-offs for Enterobacteriaceae in EUCAST 2016, that is, MIC 2µg/mL as sensitive.

Results: The study included 150 strains of Carbapenem resistance strains from June 2018 till September 2018. These included *E.coli* 9% with 25% resistant to polymyxin, *Klebsiella* spp. 42% with 41% resistant to polymyxin, *Acinetobacter* 18% with 15% resistant to polymyxin and *pseudomonas* 8% with 8% resistant to polymyxin.

Conclusion: This suggests emerging in vitro resistance against colistin in CREs

Key Words: polymyxin resistance, Gram negative organisms

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FP-Micro-0008

EVALUATION OF MULTIPLEX PCR FOR RAPID DIAGNOSIS OF DRUG RESISTANT MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

Objective: To evaluate the multiplex PCR for rapid diagnosis of Single and Multidrug resistant tuberculosis.

Place and duration of study*:* This study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP) Rawalpindi, from January to September 2018 over a period of 8 months.

Material and Methods: Eighty-four (84) cultured positive samples were included in the study using non-probability sampling technique. The drug susceptibility test was applied on MGIT 960 system for first line anti tuberculosis drugs rifamlicin, isoniazid and ethambutol. DNA was extracted from culture samples. Finally, in order to amplify the mutation for rpo B (rifampicin), kat G (isoniazid) and embB (ethambutol), multiplex PCR performed and gel electrophoresis carried out followed by visualization of mutated bands under high illumination UV light.

Results: Among 84 isolates of MTB, 18(21%) isolates were MDR and 66(79%) were single drug resistant i-e, 17(19%) for rifampicin, 39(46%) for isoniazid and 10(11%) ethambutol. Out of 18(21%) MDR cultured positive isolates further analyzed by multiplex PCR, 11(61%) showed all three mutated bands. Out of single drug resistant i-e. 39 isoniazid, 17 rifampicin and 10 ethambutol cultured positive isolates, mutated bands were detected in 28(72%) of kat G gene for isoniazid, 17(100%) of rpoB for rifampicin and 4(40%) of emb B for ethambutol in multiplex PCR. The sensitivity of Multiplex PCR for MDR, isoniazid, rifampicin and ethambutol were 68%, 85%, 80% and 60% respectively and specificity value were 100% each respectively. The positive predictive value were 100% each and the negative predictive value were 42%, 36%, 57%and 50% respectively. The diagnostic accuracy (DA) for MDR, isoniazid, rifampicin and ethambutol were 83%, 82%, 87% and 71% respectively.

Conclusion: The study revealed that Multiplex PCR is rapid and reliable method for diagnosis of drug resistant tuberculosis with high sensitivity and specificity.

Key Words: Multiplex PCR, Drug resistance, Tuberculosis

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FP-Micro -0009

MULTIPLEX PCR FOR THE DETECTION OF NEISSERIA GONORRHOEAE AND THE QUINOLONE RESISTANCE GENE

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ABSTRACT

Objective:

1. To detect *Neisseria gonorrhoeae* from the Urine of a person suspected to be infected with *Neisseria gonorrhoeae*.
2. The simultaneous detection of the Quinolone Resistant Determinant Region (QRDR) by Polymerase Chain Reaction.

Study Design: It was cross sectional study.

Place of study: This study was conducted at the department of microbiology Army medical college/National university of medical sciences.

Material and Methods: Urine sample of male patients with active urethral discharge were collected in a plastic container for simultaneous detection of *Neisseria gonorrhoeae* and quinolone resistance gene on multiplex PCR. Quality control procedures were implemented at all steps. Forward primer gyrA –W designed to include the most conserved region in gyrA sequence that is not common in non *Neisseriagonorrhoeae* species (5”GCGATTCCGC AGTTTACGA3”) was used. The gyrA-M forward primer designed to include the gyrA sequence harboring S91 and D95 mutations (5”TACCAC CCCCACGGCGATTT3”) which was used for quinolone resistance determination. These primers were paired with common reverse primer gyrA-R (5”5CGAAATTTTGCGC CATACGGACGAT3”). DNA extraction and PCR were conducted according to manufacturer’s guidelines.

Results: *Neisseria gonorrhoeae* were positive in 40% patients of urethral discharge. The Quinolone Resistant Determinant Region (QRDR) were detected in 70.8% cases of *Neisseria gonorrhoeae.*

Key Words: QRDR (Quinolone Resistant Determinant Region), PCR (polymerase chain reaction),

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FP-Micro -0010

PHENOTYPIC IDENTIFICATIONOF AmpC β-LACTAMASE PRODUCING GRAM NEGATIVE RODS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN ISOLATED FROM ICU OF A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: Over the past few years, there has been a continuous increase in the incidence of Multidrug resistance. Among multidrug Resistant Organisms, Gram Negative Rods (MDR-GNRs) have been considered as one of the most rampant agents of infections in individuals. This study determined the frequency of AmpC β-lactamase producing GNRs isolated from clinical samples of Intensive care unit patients in our setup.

Material & Methods: The current study was conducted in Department of Microbiology, AFIP; Rawalpindi from April 2018- October 2018. Gram Negative Rods (GNRs) cultured from different clinical specimens were identified by standard microbiological methods (Colony Morphology, Gram's stain, Catalase Test, Oxidase Test, Motility Test and by using API 20 E (Biomerieux, France). Screening of isolates for AmpC β-lactasmases was done by Cefoxitin 30µg disc followed by confirmatory double disc synergy test duplicated with ceftazidime disc and cefotaxime disc, using cloxacillin disc as an inhibitor.

Results: Out of 196 total clinical isolates, 99 were screened positive (50.5%). Out of screen positive isolates n=20 isolates were confirmed as AmpC producers (10.2%). 100% of resistance was observed in Ampillicin, Augmentin and Ceftriaxone, 15% in Imipenim and Cefepime, 20% in Doxycycline, 30% in Ciprofloxacin, 40% in Amikacin and Gentamicin, 10% in Tazobactam-pipracillin, 50% in Co-trimoxazole in and None of isolate was resistant to Polymixin and Tygecycline.

Conclusion: The present study highlights low burden of AmpC in a tertiary care hospital. In house antibiotic policy, infection control and epidemiological surveys will help us in controlling these resistant bugs

Key Words: AmpC β Lactamase, Gram Negative rods, Antibiotic susceptibility.

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FP-Micro -0011

DIAGNOSTIC ACCURACY OF CRYSTAL VIOLET DECOLORIZATION ASSAY FOR RAPID DETECTION OF ISONIAZID AND RIFAMPICIN RESISTANCE IN AFB SMEAR POSITIVE PULMONARY SPECIMENS

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ABSTRACT

Objective: To evaluate the diagnostic accuracy of crystal violet decolourisation assay for rapid detection of isoniazid and rifampicin resistance in AFB smear positive pulmonary specimens keeping MIT 960 as gold standard method.

Material & Methods: The smear positive pulmonary specimens after digestion and decontamination procedure were inoculated on 3 tubes, first tube was labelled as growth control (GC) with no drug, second and third tubes contained isoniazid and rifampicin at a concentration of 0.125µg/ml and 0.5µg/ml, respectively. After bacterial inoculation, the samples were incubated at 37ºC. Then 100 µL of CV (25 mg/L stock solution) was added to all the tubes after fourteen days of incubation. The tubes were further incubated for an additional 72-96 hrs. CV (blue/purple) is decolourised in the presence of mycobacterial growth; those tubes which got decolourized were reported as resistant and those which failed to decolorize were sensitive to the respective drug.

Results: Out of 50 specimens, results of 47 were reportable, 2 were contaminated and 1 yielded growth of MOTT. Average time to positivity was 15 to 20 days. 32 specimens were sensitive to INH and RIF both, 4 were MDR, 8 were resistant to INH only and 3 were resistant to RIF only. Sensitivity of INH and RIF was 83.33% and 77% respectively. Specificity of INH and RIF was 97.14% and 100%, respectively. PPV of INH and RIF was 90.9% and 100 % respectively. NPV of INH and RIF was 94.44 and 95.24, respectively. Diagnostic accuracy of INH and RIF was 93.6 % and 95.7 %, respectively.

Conclusion: Our study highlights the utility of CVDA as a rapid, inexpensive and reliable method of antibiotic susceptibility testing, however this method is laborious and there are high chances of contamination of specimens as compared to the automated systems.

Key Words: Crystal Violet Decolorization Assay, Rifampicin, Isoniazid

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FP-Micro -0012

XDR *KLEBSIELLA PNEUMONIAE* EMERGING AS A SUPERBUG IN OUR TERTIARY CARE SETTING

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ABSTRACT

Objectives: With the alarming increase of antibiotic

resistance, the world is on the cusp of Post Antibiotic Era. Resistance to Colistin is increasingly been reported among carbapenem – resistant *Enterobacteriaceae*. This study was undertaken to determine the frequency of Multi Drug Resistant (MDR), Extensively Drug Resistant (XDR) and Pan Drug Resistant (PDR) *Klebsiella pneumoniae* from various clinical specimens in our tertiary care setting.

Material & Methods: The present study was conducted in the Microbiology Department, Armed Forces Institute of Pathology (AFIP), Rawalpindi from 6th Nov 2016 to 5th May 2017. *Klebsiella pneumoniae* recovered from various clinical samples submitted at AFIP were tested for 16 antibiotics by Kirby Baeur disc diffusion method. Colistin MICs were performed by Vitek 2 systems Version 08.01.

Results: A total of 150 various clinical samples yielding growth of *Klebsiella pneumoniae* were recovered. All (100%) of the isolates were Multi drug resistant (MDR), among them 81% of the isolates were Extensively drug resistant (XDR). All 100% of the isolates were sensitive to Colistin, 84.8% isolates were sensitive to Fosfomycin. However, only 28% isolates were sensitive to Carbapenems. None of the isolate was susceptible to Ceftriaxone indicating very high antibiotic resistance.

Conclusion: Our study highlights the emergence of very high antibiotic resistance. Only acquisition of one more resistant gene for colistin will render all isolates PDR leaving no treatment option for the clinicians. Colistin resistance have already been reported worldwide which shows we are losing the battle against microbes. It is the need of the hour to follow policies for antimicrobial stewardship with true spirit to control antimicrobial resistance in our country.

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FP-Micro -0013

VARYING PATTERNS IN ANTIBIOGRAMS OF SALMONELLA ISOLATES AT TERTIARY CARE HOSPITAL HYDERABAD

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ABSTRACT

Objective: To analyze the antibiotic sensitivity pattern of salmonella-typhi.

Study Design: Retrospective study.

Material and Method: This study was conducted at Department of Microbiology LUMHS, Hyderabad from January 2018 to June 2018, in which blood samples were collected from 519 patients reported with the symptoms of typhoid fever. The age group was between neonate and 42 years. The antibiotic susceptibility test was performed by the disc diffusion method in accordance with the guidelines of Clinical and Laboratory Standards Institute, plates were incubated at 37°C, and then the antibiotic disc incorporation and zone of inhibition were measured in millimeters after 24 hours of incubation. A total of 7 different antibiotic discs were used to check the susceptibility and resistance of the clinical isolates obtained. Based on the zone of inhibition the isolates were classified into sensitive, intermediate, and resistant pattern.

Results: The antibiotic sensitivity patterns against Salmonella-typhi were investigated using seven antibiotics such as, amplicillin, azithromycin, cefixime, ceftriaxone, ciprofloxacin, meropenem, and sulphome-thaxazole / trimethoprim. This study shows 86.70% resistance, and 13.30% sensitive for amplicillin; 76.50% resistance, and 23.50% sensitive for cefixime; 72.20% resistance, 21.90% intermediate, and 5.90% sensitive for ciprofloxacin; 87.84% resistance, and 12.16% sensitive for sulphomethaxazole/ trim; 2.57% resistance, 0.43% intermediate, and 97% sensitive for azithromycin; 77.07% resistance, and 22.93% sensitive for ceftriaxone, and most sensitive against meropenem with 99.42%.

Conclusion: This study shows that the bacterium salmonella typhi is most sensitive to the antibiotic Meropenem followed by Azithromycin, Cefixime, Ceftriaxone, Ampicillin, Sulphomethaxazole/ trimethoprim and least sensitive and most resistant to Ciprofloxacin.

Keywords: Sensitivity, Intermediate, Multi-drug resistant.

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FP-Micro -0014

SIX YEAR EVALUATION OF ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *SALMONELLA ENTERICA* SEROVARS TYPHI AND PARATYPHIA IN A TERTIARY CARE HOSPITAL, PAKISTAN

Sana Jamil

ABSTRACT

Objective: To determine trends and antibiotic susceptibility patterns of Salmonella *enterica* serovars Typhi and Paratyphi A over 6 years in a tertiary care hospital in Karachi, Pakistan.

Study design: Retrospective cross sectional.

Material and Method: From January 2013 to August 2018, a total of 201,414 blood culture specimens were collected from patients belonging to all age groups and submitted to the clinical laboratory. Isolates of *Salmonella enterica* serovars Typhi and Paratyphi A were identified by standard microbiological and biochemical procedures.

Results: The sensitivity data of 428 positive isolates of *S.* Typhi and *S.* Paratyphi A isolated during the six-year period were reviewed. The majority of isolates were *S.* Typhi (67.2%). Over six years, the incidence of multidrug-resistant (MDR) *S.*Typhi showed a rising trend, ranging from 23.68% to 59.25%, while none of the *S.* Paratyphi A were found to be MDR except for the year 2017 with an incidence of 5.5%. Dramatic increase in Fluoroquinolone resistance has been seen from 10.5% to 94.1% for *S.typhi* and 12.5% to 90.9% for *S. paratyphi A* in six years. Ceftriaxone-resistant *S.* Typhi started to appear in year 2017 reaching incidence as high as 6.25% and dramatically inclining to an incidence of 43.13% in August 2018.

Conclusion: We report a high rate of multidrug and fluoroquinolone resistance. This can have implications for empiric antimicrobial therapy in a country endemic for typhoid. We also report an emergence of XDR *S.typhi* along with a rising trend which is a startling demonstration of how a ubiquitous antibiotic resistance plasmid can be acquired by MDR *S. Typhi*, rendering it XDR and further narrowing treatment options.

Key Words: Multidrug resistance, Extensive drug resistance, *Salmonella* typhi, Paratyphi.

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FP-Micro -0015

ROLE OF ASCORBIC ACID ON BIO-FILM FORMATION OF *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Objective: To study the effects of and its role in prevention of bio-film formation.

Place and duration of study: The study was carried out at the Department of Microbiology, Pathology Department, PNS Shifa Karachi.

Study Design: Observational analytical.

Material and Methods: ATCC 25873 strain of *Pseudomonas aeruginosa* was selected for the study and the organism was cultured in varying concentrations of glucose and examined every day for the formation of biofilm. At the same time the same strain was inoculated in concentration of glucose along with 1,2,4,8 and 16 ug /ml of ascorbic acid and incubated at 35 C for seven days examining the growth every day. Also preformed biofilm strain was incubated along with different concentrations of ascorbic acid. The biofilm formation was confirmed by scanning electron Microscopy

Results: After 48 hours biofilm formation was noted in the tubes without any ascorbic acid while no biofilm was formed in ascorbic acid containing tubes even after seven days.

Conclusion: It has been confirmed that low concentrations of vitamin C can be effectively used as a pre-treatment or a combined treatment to destabilize bacterial biofilms. Further, study is in progress.

Key Words: Ascorbic acid, Biofilm, *Pseudomonas aeruginosa.*

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FP-Micro-0016

RISING TREND OF CEFTRIAXONE RESISTANT TYPHOIDAL *SALMONELLAE* IN SIND

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ABSTRACT

Objective: To determine the trend of ceftriaxone resistance in typhoidal *Salmonellae* in Sind.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: The study is being carried out in the Department of Microbiology, PNS Shifa Hospital, Karachi, from July 2018 and is still continued.

Material & Methods: Blood culture samples received from the wards and outpatient departments were included. Isolates of Salmonella were dealt with standard microbiological procedures. The antimicrobial sensitivity against the typhoidal Salmonellae was determined using Kirby-Bauer disc diffusion method in accordance with the guidelines of Clinical and Laboratory Standards Institute (2018).

Results: A total of 176 isolates of *Salmonella typhi* have been isolated. Antimicrobial sensitivity pattern of *Salmonella typhi* reveals 30.68% isolates to be ceftriaxone resistant while 69.31% isolates turned out to be Ceftriaxone sensitive.

Conclusion: There is an alarming increase in ceftriaxone resistant isolates of typhoidal salmonellae in our region. These isolates are only found to be sensitive to macrolides and carbapenems. This situation leaves us with very limited choice of antimicrobials against typhoidal salmonellae. Moreover, the antimicrobial sensitivity of typhoidal Salmonellae to conventional agent continues to deteriorate. Antimicrobial stewardship and infection control practices are the only solution of this galling situation.

Key Words: Antimicrobial resistance, *Salmonella typhi*, typhoid

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MICROBIOLOGY POSTERS PRESENTATION ABSTRACTS

P-Micro-0001

FREQUENCY OF CARBAPENEM RESISTANCE AMONG THE CLINICAL ISOLATES OF *ACINETOBACTER BAUMANII*

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ABSTRACT

Objective: *Acinetobacter baumanii* is one of the most common organisms causing nosocomial infections. It has gained more attention because of its prolonged environmental survival and tendency to develop drug resistance. The objective of our study is to determine the carbapenem resistance among the clinical isolates of *Acinetobacter baumanii*

Study Design: Descriptive cross-sectional.

Study setting and duration: The study was carried out at department of microbiology AFIP, Rawalpindi from July 2017 to July 2018.

Material and Methods: Clinical samples were inoculated on Blood agar and MacConkey Agar and incubated at 35 ± 2°C. Growth is observed next day and organism is identified by Gram staining, catalase, Motility, Biochemical testing using API 20 NE. Antimicrobial susceptibility testing of the isolates for imipenem (30µg) and meropenem (30µg) was carried out by Modified Kirby Bauer disk diffusion method according to CLSI guidelines. Isolates found resistant to either imipenem or meropenem or both were taken as carbapenem resistant. *Escherichia coli* ATCC 25922 was used as quality control strain.

Results: Out of 470 clinical samples yielding growth of *Acinetobacter baumanii* 415(88%) belongs to males and 55(12%) to females. Various samples yielding the growth of *Acinetobacter baumanii*, includes; Respiratory specimens (NBL, EB washing, Sputum) 60%, pus 16%, pus swab 15% and Blood 9%. Among all 90% were found resistant to carbapenems**.**

Conclusion: Clinical isolates of *Acinetobacter baumanii* showed very high resistance to carbapenems. Therefore, carbapenem should not be used as empirical treatment when *Acinetobacter baumanii* is suspected as the cause of infection.

Key Words: *Acinetobacter baumanii*, Carbapenem resistance

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P-Micro-0002

SUSCEPTIBILITY OF NITROFURANTOIN AND FOSFOMYCIN AMONG URINARY ISOLATES OF *ESCHERICHIA COLI*

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ABSTRACT

Objectives: To determine antimicrobial susceptibility of nitrofurantoin and fosfomycin among urinary isolates of *Escherichia coli*.

Study design: Descriptive cross-sectional study.

Study duration: The study was conducted at Department of Microbiology AFIP Rawalpindi from May 2018 to September 2018.

Material & Method: Mid-stream urine specimens were inoculated on BLOOD agar and CLED agar and incubated at 35±2° C . Growth was observed and *Escherichia Coli* was identified by Gram staining, Catalase, Motility test and API 20E(Bio murex) as per standard procedure. Antimicrobial susceptibility testing of isolates for Nitrofurantoin and Fosfomycin were carried out by Modified Kirby-Bauer disc diffusion method according to CLSI guidelines. ATCC 25922 *Escherichia coli* was used as quality control strain.

Results: Total 400 samples yielded the growth of *Escherichia coli,* out of which 180 were male and 220 were female samples. Among males, 14(7.77 %) were resistant to nitrofurantoin and 1(0.55%) were resistant to fosfomycin. Among females, 9(4.09%) were resistant to nitrofurantoin and 6(2.72%) were resistant to fosfomycin. Among age groups, below 45 years, 6(4.76%) were resistant to nitrofurantoin and 2(1.58%) were resistant to fosfomycin. Between 46- 66 years, 4(2.81%) were resistant to nitrofurantoin and 3(2.11%) were resistant to fosfomycin. Between 67 -90 years, 17(12.87%) were resistant to nitrofurantoin and 4(3.03%) were resistant to fosfomycin.

Conclusion: Fosfomycin and Nitrofurantoin showed good susceptibility in urinary isolates of *Escherichia coli* and can be used empirically in our setup.

Key Words: Nitrofurantoin, fosfomycin, *Escherichia coli*, urinary isolates.

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P-Micro-0003

FREQUENCY OF CARBAPENEM RESISTANCE AMONG THE CLINICAL ISOLATES OF *KLEBSIELLA PNEUMONIAE*

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ABSTRACT

Objective: *Klebsiella pneumoniae* is considered as one of the most serious infectious agents specially in hospital settings. This bug is now becoming resistant to many classes of antimicrobials including carbapenems. Carbapenems are commonly used as empirical therapy specially in ICUs. The objective of this study is to determine the frequency of carbapenem resistance among the clinical isolates of *Klebsiella pneumonia*.

Study design: Descriptive cross-sectional.

Study setting and duration: The study was done at department of Microbiology AFIP Rawalpindi from July 2017 to June 2018.

Materials and method: Various clinical samples were inoculated on Blood agar and MacConkey Agar and incubated at 35 ± 2℃. Growth was observed and *Klebsiella pneumoniae* was identified by Gram staining, motility and Biochemical testing using API 20 E (Biomurex). Antimicrobial susceptibility testing of the isolates for imipenem (30µg) and meropenem (30µg) was carried out by Modified Kirby Bauer disk diffusion method according to CLSI guidelines. Isolates found resistant to either imipenem or meropenem or both were taken as carbapenem resistant. ATCC 25922 *Escherichia coli* was used as quality control strain.

Results: Out of 700 samples yielding growth of *Klebsiella pneumonia,* 480(68%) belongs to males and 220(32%) to females. Various samples yielding growth of *Klebsiella pneumoniae* includes; urine 29%, pus 16%, respiratory specimens (NBL, BAL, EB washings, Sputum) 35%, pus swab 11% and Blood 9%. Among all 60% isolates were found resistant to carbapenems.

Conclusion: *Klebsiella pneumoniae* showed high resistance to carbapenems, hence their use as empirical therapy should be limited specially in hospital settings when *Klebsiella pneumonia* is suspected as the cause of infection

Key Words: Carbapenem, Antimcrobial resistance, *Klebsella pneumonia.*

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P-Micro-0004

BACTERIOLOGICAL EXAMINATION OF DRINKING WATER IN RAWALPINDI

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ABSTRACT

Objective: The objective of this study was to assess bacteriological quality of drinking water in Rawalpindi-Pakistan.

Study Design: Cross-sectional descriptive study.

Place and Duration of study: Study was conducted at Department of Microbiology, Armed Forces Institute of pathology, Rawalpindi from July 2018 to Sep 2018.

Materials and Methods: All water samples submitted at AFIP from Rawalpindi and surrounding areas for bacteriological examination of water were collected in sterile bottle under aseptic conditions. The specimens were inoculated on MacConkey agar after being filtered through Milipore water testing system using 0.22 µm filter as per manufacturer instructions. The isolates were then identified by conventional methods. Coliforms were identified by lactose fermentation, Colony morphology, Gram stain, Motility, Catalase, Oxidase and Indole test. API-20E was utilised for Indole positive colonies to confirm the presence of *Escherichia coli*. Results were interpreted according to WHO criteria for drinking water.

Results: A total of 462 water samples were analysed, out of which 164 (35.5%) were declared unsatisfactory and the rest 298 (64.5%) were found satisfactory for bacteriological examination of drinking water. Out of 462 samples 362 (78%) were treated water samples i.e filtered/chlorinated and 100 (21%) were untreated. Out of 362 treated samples 97(26%) were found satisfactory. While out of 100 untreated samples (67%) were found unsatisfactory.

Conclusion: A high frequency of polluted water samples indicates that bacterial contamination is significant problem in Rawalpindi. Chlorination, establishment of water filtration plants and regular monitoring and proper maintenance of already established filter plants can improve this situation.

Key Words: Drinking water, Bacteriological examination.

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P-Micro-0005

GENOTYPIC CHARACTERIZATION OF EXTENDED-SPECTRUM B-LACTAMASES (ESBLS) PRODUCERS AMONG BURN CLINICAL ISOLATES FROM TERTIARY CARE HOSPITAL LAHORE, PAKISTAN

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ABSTRACT

Objectives: The objectives of study include observing the efficacy of antibiotics being used to treat post-burn infections, phenotypic and molecular detection of ESBLs producing bacteria.

Study Design: The descriptive case-series study was conducted from June 12, 2017 to June 12, 2018 in Jinnah hospital's burn and reconstructive surgery center Lahore. The sampling strategy was based on purposive non-probability. A total of 150 post-burn infected patients were included in the study.

Materials and Methods: The clinical specimens included burn wound swab and urine. The isolation of the bacterial pathogens was performed on differential and selective media. The antimicrobial sensitivity testing was performed

on Mueller Hinton's agar according to CLSI guidelines 2017. The cephalosporins resistant strains were further processed for the phenotypic and genetic analysis. Phenotypic detection of ESBLs was performed by combination disk test (CDT), double disk synergy test (DDST). Molecular detection of *bla*OXA, *bla*SHV, and *bla*TEM genes was performed using multiplex PCR.

Results: 125 of the clinical specimens were positive for cephalosporins resistance which was observed by antimicrobial sensitivity testing. All of the isolates were Gram negative and *Pseudomonas* spp., was the most prevalent (48%) followed by *Klebsiella* spp., *Acinetobacter* 20%), and *Proteus* spp., (9%). Phenotypic detection by CDT and DDST proved to be helpful for the detection of ESBLs 12% and 15% of the isolates. Molecular detection of ESBLs genes multiplex PCR proved to be helpful for the detection in 36% of bacterial strains with the *bla*TEM, 19% strains with *bla*OXA, and 10% with *bla*SHV genes.

Conclusions: Antimicrobial resistance by Gram negative bacterial isolates against cephalosporins is increasing in post-burn patients. Phenotypic tests including CDT and DDST proved least effective. Double disc synergism test (DDST) is more effective than combination disc test (CDT) for the phenotypic detection of ESBLs. Molecular detection of ESBLs producing bacteria by Multiplex PCR is the most effective method for rapid diagnosis.

Keywords: Burns, MDRs, ESBLs, *bla* TEM, Lahore, Pakistan.

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P-Micro-0006

UPDATE ON DIAGNOSIS, PATHOPHYSIOLOGY AND MANAGEMENT OF ACANTHAMOEBA KERATITIS

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ABSTRACT

Objective: Clinico-pathological evaluation and management of Acanthamoeba keratitis

Material and Methods: This study includes four cases of acanthamoeba keratitis (AK) from Dec 2016 -July 2018. All cases had received multiple treatments at other paces without alleviation of the symptoms. All cases were carefully observed by slit-lamp examination. The classification of the disease was done by the clinical progression of each case with due consideration of literature. A combination therapy of Chlorhexidine 0.02%; PHMB 0.02%; Natamycin 5% and Vigamox 0.3% was given during a period of 6-10 weeks along with oral Itraconazole 100 mg for 2 weeks and Vibramycin 100 mg for 6 weeks. The patients were initially admitted for three days and then follow up was done weekly till recovery in patient's vision & improvement in pain. The dosage and combination therapy were adjusted in accordance with individual response.

Results: All four cases were males with ages between 20-45 years, showed common feature of severe pain, visual loss (hand movement), ring ulcers and progressive inflammatory infiltrate. All cases showed improvement in pain with halting of progression with healing of corneal ulcer and gradual recovery in vision from hand movement (HM) to 6/36. Faint corneal opacification and vascularization remained in all cases.

Conclusion: Acanthamoeba is a protist that causes keratitis in humans. AK is a progressive, infiltrative, melting, perforating/ cicatricial form of keratitis. Early diagnosis and aggressive treatment may be able to reduce ocular morbidity and blindness.

Key Words: Acanthamoeba keratitis; combination therapy, ring ulcer, melting keratitis.

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P-Micro-0007

FREQUENCY AND ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA* SPECIES IN PATIENTS WITH SUSPECTED CANDIDIASIS

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ABSTRACT

Objective: Among fungal diseases *Candida* species are the most common fungal pathogens, responsible for both superficial and systemic fungal infections. In candidiasis, *Candida albicans* is the most prevalent in diseased as well as healthy population. The objective of current study was to estimate the prevalence of *Candida* species and its susceptibility to antifungal drugs.

Material and methods: Total 686 samples from Jan-2016 to Oct-2017including from respiratory tract, urogenital tract, gastrointestinal tract, pus, tissue and body fluids were analyzed for fungal isolation by culturing at Sabouraud Dextrose Agar, fungal identification by API C AUX and Minimum Inhibitory Concentration of antifungal drugs by E-test.

Results: Out of 686 specimens, 111 (16%) were positive for *Candida* species. *Candidaalbicans*70 (63%) was most dominant while frequency of non-albican species was *Candidatropicalis* 19 (17%), *Candidaparapsilosis* 8 (7%), *Candidakrusei*4 (4%), *Candidaglabrata* 9 (8%), *Candidarugosa*1 (1%).Overall resistance was 18%, 12%, 11% and 20% against Fluconazole, Itriconazole, Voriconazole and Amphotericin B respectively. *Candidakrusei* and *Candidarugosa* were 100% resistant to fluconazole while *Candidagalabrata* was 100% resistant to Itraconazole.Voriconazole was found to be least resistant and choice of drug among azole group.

Conclusion: Identification of *Candida*upto species level and to perform antifungal susceptibility profiling is necessary to reduce antifungal resistance and for improved clinical care.

Key Words: Mycology, Candida, Antifungal, Infection

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P-Micro-0008

FREQUENCY AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF *CANDIDA* SPECIES IN DIFFERENT CLINICAL ISOLATES

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ABSTRACT

Objective: From last few decades Candida species emerging as major pathogenic organism involving in human diseases, especially in nosocomial infections, immunocompromised and patients with hematological malignancies. There are considerable morbidity and mortality due to underlying fungal diseases and researcher itemized that fungal disease is an important public health problem. The objective of this study was to determine frequency and antifungal susceptibility pattern of *Candida* species in different clinical isolates.

Material and Methods: A total of 220 clinical samples were collected during this descriptive study. Clinical samples include urine, high vaginal swabs, blood, tracheal aspirate, sputum, CVP lines and pus. All clinical samples were process in safety cabinet applying standard microbiological technique. The *Candida* species were identified either on conventional technique or by API system. The antifungal susceptibility testing was performed by disc diffusion method on Mueller Hinton agar, 2% glucose with methylene blue.

Results: Out of 220 clinical samples urine samples were 82 (37.3%), high vaginal swabs 94 (42.7%), sputum 21 (9.5%), tracheal aspirates 8 (3.6%), Blood 10 (4.5%), CVP line 3 (1.4%) and pus 2 (0.9%). The result of susceptibility testing determined by agar diffusion methods shows that 91.8% isolates were sensitive to Amphotericin B, while 8.2% were resistant. 40.9% percent isolates sensitive to fluconazole, 57.7% resistant and 1.4% were intermediate. 45% isolates were sensitive to Intraconazole 54.5% were resistant and 0.5% were intermediate. 41.4% isolates were sensitive to Voriconazole 57.3% were resistant and 1.4% were intermediate. 31.4% isolates were sensitive to clotrimazole and 68.6% were resistant.

Conclusion: The development of antifungal resistant especially in triazole in Candida species is flattering a severe therapeutic problem globally. Mostly these organisms are intrinsically resistant to some triazoles, but also observe the increase to Amphotericin B and other essential antifungals. There is strongly recommended the antifungal susceptibility and active surveillance of antifungal drugs for the reducing of emergence of resistant in such antifungals and provide the data to the clinician for treating such organisms.

Key words: Amphotericin B, antifungals, Candida, Azole, Methyline blue

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P-Micro-0009

TO OBSERVE PREVALANCE OF MANTOUX TEST IN SINDH

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ABSTRACT

Objectives: The aim of this study was to find out the prevalence of Mantoux test in Sindh.

Study Design: Cross-Sectional Descriptive Study

Material and Methods: This Cross-Sectional Descriptive Study was carried out at various branches of Diagnostic & Research Laboratory LUMHS in Sindh from Jan 2018 to Sep 2018. Mantoux test is performed by injecting a standard dose of 5Tuberculin units (0.1ml) into the skin intradermally. The result should be read between 48-72 hours after the test and the indurated area is measured in millimeter units, the result is considered as below 10 mm as negative and above 10 mm as positive. The skin reaction following Mantoux test is a form of cell mediated delayed hypersensitivity (Type IV) reaction of the skin. The whole process take place up to 72 hours and the reaction is completed by formation of skin induration.

Results: A total of 3121 patients prescribed by physician's clinically and on the basis of symptoms of pulmonary tuberculosis like fever, productive cough and weight loss were included. The mean age of the patients was 14.6 years with range from one year to 95 years old. There was a bit male dominance in the study sample with 1614 (51.7%) males and 1507 (48.3%) female patients in the study sample. From total 3121 patients 2805 (89.9%) were negative and 316 (10.1%) were positive, from 316 positive patients 185 were females and 131 male patients and mostly the belongs to big cities from Hyderabad &Jamshoro were 223, Mithi 62, Sukkur14, Mirpurkhas 4and remaining small cities were Kandiaro 6, Tando Adam 4, Kandhkot 2 &TandoAllahyar 1

Conclusion: It was stated that Mantoux test has some role in diagnosis of tuberculosis.

Key Words: Mantoux, Tuberculosis, Hypersensitivity

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P-Micro-0010

ANTI-MICROBIAL SUSCEPTIBILITY PROFILE OF MDR ACINETOBACTER AGAINST TIGECYCLINE

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ABSTRACT

Objective: Antimicrobial resistance in MDR Acinetobacter against last resort antibiotics like Tigecycline has been reported. The objective of this study was to determine

antimicrobial resistance of Multi Drug Resistant *Acinetobacter* against Tigecycline in clinical isolates yielded from intensive care unit patients of a tertiary care hospital.

Material and Methods: This descriptive study was conducted in Medical Intensive Care Unit of Holy Family Hospital. Hospital records for a period of 1 year from April 2017 to April 2018 were analyzed. A total of 396 clinical isolates from blood samples, tracheal suction catheter tips, Endotracheal tube tips, Dialysis catheter tips and CVP line catheter tips were included using consecutive sampling technique. Antimicrobial profile of Multi-Drug Resistant Acinetobacter against Tigecycline was noted. Data was analyzed using SPSS v22.0. Descriptive statistics were applied.

Results: From the included time period, 396 isolates, 191 (48.2%) isolates were obtained from female while 205 (51.8%) from male patients were found to be culture positive for MDR Acinetobacter.

A total of 391 (98.7%) isolates were susceptible while 5 (1.3%) isolates were resistant to Tigecycline.

Conclusion: Increasing Anti-microbial resistant of Multi Drug Resistant clinical isolates of Acinetobacter against Tigecycline is alarming.

Key Words: Multi Drug Resistance, Intensive Care Unit, ICU, Acinetobacter, Tigecycline.

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P-Micro-0011

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION OF ANTIMICROBIAL AGENTS USED AGAINST DRUG RESISTANT KLEBSIELLA STRAINS

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ABSTRACT

Objective: Multi drug resistance has now become a worldwide therapeutic challenge due to the widespread use of broad-spectrum antibiotics. Klebsiella species have significant importance in clinical field as they cause various infections in human and are considered as potential pathogens that express antibiotic resistance through their strong enzymatic activity. The present study was carried out to determine the minimum inhibitory concentrations by Macro broth dilution method in Klebsiella species for implementing the judicial use of drugs and proper therapy.

Material and Methods: 125 Klebsiella strains were collected for this study and the minimum inhibitory concentrations (MIC) were determined using eight representative antibiotics by Macro broth dilution Method.

Results: It was found that 102 Klebsiella isolates were resistant to Gentamicin having an MIC value ≥16 µg/ml, 102 to Cephradine having an MIC value ≥8 µg/ml, 100 to AMC having an MIC value ≥32 µg/ml, 79 were resistant to Cefuroxime having an MIC value ≥32 µg/ml, 45 to Ciprofloxacin having an MIC value ≥4 µg/ml, 36 were resistant to Cefotaxime having an MIC value ≥4 µg/ml, 32 were resistant to Cefoperazone having an MIC value ≥ 64 µg/ml, 10 were resistant to Imipenem having an MIC value ≥ 4 µg/ml. It has been identified that Klebsiella strains are highly resistant against gentamicin, cephradine, co-amoxyclav and cefuroxime. The most effective antibiotics for Klebsiella infection were found to be imipenem.

Conclusion: The prevalence of ESBL among clinical isolates varies greatly worldwide and the patterns are changing over time. There is a need to formulate a policy for empirical therapy in high risk units where infection due to resistant organism is much higher. The knowledge and awareness of the resistance pattern of the microorganisms is necessary for the judicial use of antibiotics. An appropriate antimicrobial therapy can only be started timely with the early detection of resistance pattern either phenotypically or genotypically. Phenotypic tests will hold significance when the facilities of molecular methods are not available.

Key Words: MIC, ESBL, Klebsiella, Macro broth dilution

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P-Micro-0012

ROLE OF PHENOTYPIC SWITCHING IN STABILITY AND PERSISTENCE OF PSEUDOMONAS AERUGINOSA BIOFILMS

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ABSTRACT

Objective: This study was designed to explore the role of different phenotypes of *P. aeruginosa* in the development, stability and persistence of biofilm.

Material and Methods: A total of seventeen (17) waterborne biofilm producing strains of *P. aeruginosa* were studied. These isolates were identified on the basis of typical phenotypic characters, i.e. growth on cetrimide agar and by amplification of 16S rDNA. Tube method was used for development of biofilms on glass slides and growth and exopolysaccharides production was measured after18h, 24h, 36h, 48h, 72h and 96h of incubation. The Crystal violet assay was used for quantification of biofilms. Population and phenotypic variance were studied by the drop plate method. The hydrophobicity of strains was evaluated by the bacterial adhesion to a polar solvent test.

Results: Study showed that the subject isolates of *P. aeruginosa* adopted a biofilm life style after 36h of incubation at 35°C. After 24h the adhesion started, but it was reversible and easily dispersed by simple washing. However, after 36h the irreversible adhesion, difficult to disperse, was noticed. The biofilm consortia harbor three different phenotypes: i. wild types, showed typical *P. aeruginosa* characters on Cetrimide agar; ii. Slow growers, showed poor pigmentation and take >36h for colony development, and iii. Small colony variants (SCVs) are metabolically inactive very slow growing and producing

pinpointed non pigmented colonies. Interestingly, increase of incubation time of biofilm consortia results in strong adhesion and dominance of SCVs. Comparative analysis showed that these phenotypes i.e. SCVs were highly hydrophobic and persistent in biofilm consortia due to the production of excessive amounts of exopolysaccharides.

Conclusion: This study showed that phenotypic heterogeneity is a characteristic feature of *P. aeruginosa* biofilms and all of these phenotypes have a major role in stability and persistence of biofilm consortia.

Key Words: Biofilms, Hydrophobicity, *P. aeruginosa*, Phenotypes, SCVs

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Micro-0013

PREVALENCE OF MULTI DRUG RESISTANCE IN KLEBSIELLA PNEUMONIAE AT TERTIARY CARE HOSPITAL HYDERABAD

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ABSTRACT

Objective: The Gram-negative bacterium *Klebsiella pneumoniae* is widely distributed in the environment and increasingly reported as a cause of invasive infections in healthcare settings, particularly in immunocompromised patients. Antimicrobial resistance in *K. pneumonia*e is increasing, particularly beta-lactamases and carbapenemases having been well-characterized as increasing the infection threat. The objective of this study was to determine the prevalence of multi drug resistance in *klebsiella pneumoniae*.

Material and methods: This retrospective study was conducted at diagnostic and research laboratory LUMHS, Hyderabad for a period of 6 months from March 2018 to August 2018, 200 samples were included who were infected with *klebsiella pneumoniae*. The antibiotic susceptibility pattern was analysed by disc diffusion method on the basis of sensitive and resistance pattern.

Results: Total 200 samples were included in this study from which 90 samples were of males and 110 were females. 100 pus samples, 60 urine samples and 40 blood samples were collected from these patients and studied for antibiotic sensitivity.154(77%) patients were sensitive and 46(23%) patients were resistant to amikacin, 172 (86%)patients were sensitive and 28(14%) patients were resistant to meropenem, 72(36%) patients were sensitive and 128(64%) patients were resistant to ceftrixone, 102(51%) patients were sensitive and 98(49%) patients were resistant to ciprofloxacin, 70(35%) patients were sensitive and 130(65%) patients were resistant to sulphomethaxazole.

Conclusion: This study shows that most sensitive drug for *klebsiella peumoniae* are meropenem and amikacin, least sensitive and most resistant is sulphomethaxazole followed by ceftrixone.

Key Words: *Klebsiella pneumonia*, Multidrug resistance.

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P-Micro-0014

ANTMICROBIAL SESITIVITY PATTERN OF PSEUDOMONAS AERUGINOSA AND DETECTION OF MULTIDRUG EFLUX GENE IN PESHAWAR

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ABSTRACT

Objective: Pseudomonas aeruginosa is a common organism infecting humans, animals and plants. It is a normal flora of water and soil. This organism often invades tissues with lowered immunity. The sites mostly infected by the organism are surgical wounds, blood, urinary tract and lungs (pneumonia). In some cases the infection may be fatal. The organism is inherently resistant to many antimicrobials and has genetic capacity to develop resistance to other antimicrobials. The objective of this study was to determine antimicrobial sensitivity pattern and identify multidrug efflux MexA gene in multidrug resistant strains of Pseudomonas aeruginosa in Peshawar.

Material and Methods: Urine specimens and pus form different wounds were obtained. Media used for inoculation were blood agar and MacConkey medium. Other biochemical tests performed for confirmation were oxidase test and Triple sugar iron medium.

Results: Sensitivity tests showed that resistance to Amikacin was 24%, Co-Amoxiclave 100%, Ceftazidime 51%, Ceftriaxone 100%, Cefotaxime 100%, ciprofloxacin 17%. meropenem 13%, Polymixin B 22%, Cefperazone/ Salbactum 25%, Piperacillin/ Tazobactum 18%. In total 50 specimens of Pseudomonas aeruginosa isolates were processed for species specific O-antigen acetylase gene. The confirmed isolates were examined by conventional PCR for MexA gene. In 50 specimens, PCR was positive in 49 specimens for drug resistance multidrug efflux MexA gene and positive in 49 specimens for O-antigen acetylase gene, (done separately).

Conclusion: Meropenem showed least resistance, followed by ciprofloxacin and Piperacillin/ Tazobactum. In Multidrug resistant (MDR) Pseudomonas aeruginosa specimens, MexA gene was present in almost all sample populations of the organisms. It gives us a clue that blocking the Mex AB OprM efflux pump will reduce the resistance of the organism.

Key Words: *Pseudomonas aeruginosa*, Multidrug efflux gene

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P-Micro-0015

TO DETERMINE THE PREVALENCE AND RISK FACTORS CONTRIBUTING TO THE NONADHERENCE OF PATIENT USING ANTI-TB THERAPY IN DISTRICT MIRPUR AJK

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ABSTRACT

Objective: Non-adherence to TB treatment is the major hurdle in controlling tuberculosis. Many TB patients do not complete their entire course of anti-TB medications and do not know about the consequences of non-adherence such as MDR-TB, XDR-TB, relapse, and death. So, in the current study, we are exploring the prevalence and risk factors contributing to non-adherence of the patient using anti-TB therapy.

Study Design: This was a cross-sectional study with a quantitative approach.

Method: In this study, a sample was calculated from statistically registered patients and was selected from 5 TB centers of District Mirpur by using random sampling technique. Data were collected by using archival record (DOT registration book, patient Medical chart) and interviewing the patient by using questionnaire and analyzed by SPSS 23. The correlation was tested by using Chi-Square Test.

Result: In this study, out of 108 patients, 59% were female and 41% were males, with the mean age of 50.87 + 24.31 years. Non-adherence was found in 64%. The percentage of nonadherence was higher in females (68%) and older age group (72%). Adherence was poor (35%) in the newly diagnosed patient then registered after default (26%) and then relapsers (15%). Non-Adherence was more in the initiation phase (77%) as compared to the continuation phase (23%). Literacy rate (42%), forgetfulness (59%), length of treatment (77%), the burden of medication (70%) and traveling distance (52%) were found commonly contributing factors to non-adherence. A strong association was found between educational status, pill burden, forgetfulness, length of treatment, patient counseling, traveling distance and non-adherence.

Conclusion: It is concluded that non-adherence was higher in females, old age, newly diagnosed patient and in the initiation phase. It is recommended that non-adherence can be reduced by implementation of DOT, patient counseling by a joint team effort of experts such as pharmacist and health educator.

Keywords: Non-adherence, DOT, TB patients, District Mirpur, Tuberculosis.

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MOLECULAR PATHOLOGY SCIENTIFIC SESSION ABSTRACTS

SS-MolPath-0001

WHOLE GENOME-BASED ANALYSIS FOR DETERMINATION OF DRUG RESISTANCE AND OUTBREAK SURVEILLANCE FOR TUBERCULOSIS AND TYPHOID

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ABSTRACT

Background: The development of resistance to anti-microbial drugs in pathogens is causing additional burden due to the diseases such as tuberculosis and typhoid. The extensively drug-resistant tuberculosis (XDR-TB) has emerged as a major public health concern. XDR-TB is defined as Mycobacterium tuberculosis (MTB) resistant to at least rifampin (RIF) and isoniazid (INH) among the first-line anti-TB drugs, fluoroquinolones and to at least one injectable aminoglycoside. Resistance in MTB is associated with single nucleotide polymorphisms (SNPs) in particular genes but not all phenotypic resistance can be explained by known target genes.

Recently, XDR-typhoid was identified in Salmonella typhi strains from Sindh which were resistant to chloroamphenicol, ampicillin, trimethoprim, flouroqu-inolones and cephalosporins five antibiotics.

Material and Methods: DNA isolated from bacteria was subjected to sequencing using Illumina sequencing by synthesis method. FASTQ files were aligned with the reference genome to identify SNPs in individual strains. Phylogenetic analysis was performed using lineage association tree to determine relatedness between strains.

Results: XDR-TB strains revealed mutations in particular target genes associated with resistance to Rifampicin, Isoniazid, Pyrazinamide, Flouroquinolones and Kanamycin. However, SNPs in target genes could not explain phenotypic resistance in some strains. Here, efflux pumps were found to have SNPs; Rv0194, Rv2688c, Rv1634, drrA and drrB have been associated with drug resistance.

XDR-typhoid strains were analysed and found to contain a plasmid resistance gene conferring beta-lactamase and flouroquinolone resistance. This was found to have acquired additional resistance gene through horizontal transfer of plasmids from enteric strains.

Conclusion: Bacterial genome-based analysis provides insights into the drug resistance mechanisms of pathogenic strains and identify appropriate treatments. It can also be used to determine relatedness in outbreak investigations. Soon, both in the research and clinical laboratories we will be able to use it for rapid diagnosis of drug resistance in pathogens in routine practice.

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SS-MolPath-0002

DRUG RESISTANCE MUTATIONS IN HIV-1-INFECTED DRUG-NAIVE PATIENTS OF KARACHI, PAKISTAN

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ABSTRACT

Objective: From last two decades Pakistan has witnessed a change from low prevalence to a country with concentrated HIV epidemic in high risk groups. Prescribed antiretroviral drugs are also causing resistance and these resistant are now been circulating. Previous researchers from, Pakistan showed a very high sensitivity pattern towards antiretroviral drugs. In this study we studied drug resistance among HIV infected patients in Pakistan using Bioinformatics tools.

Study Design: Cross sectional study

Material and Methods: Whole blood samples of 50 drugs naive patients were collected form all patients. After DNA extraction, all positive samples were subjected to nested PCR using specific primers. The amplified products were sequenced and mutations in HIV sequences were analyzed and pattern Stanford University. Shannon entropy analysis was performed to determine variability in amino acid sequence of HIV-1 RT region in drug naive patients.

Result: Drug resistance analysis showed that patients were showing high level resistance to prescribed E138A and K103N respectively against NNRTI drugs, 75% of the sequences showed resistance mutation at MI84V against NRTI drugs. Shannon entropy analysis at amino acid positions p.119,p.130,p.157,p.154

Conclusion: According to our results majority of the drug naïve patients have shown major or minor mutations resulting in a drug resistant virus in our community. Our results are showing high part of the world. These results are alarming on contrary to the previous reported data in which hardly any drug resistance has been reported from this area.

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SS-MolPath-0003

CURRENT UPDATES OF NEXT GENERATION SEQUENCING IN MOLECULAR PATHOLOGY

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ABSTRACT

The rapid progression and extensive use of next generation sequencing (NGS) in clinical laboratories has allowed an incredible progress across multiple fields. Recently NGS

emerges as technique to decrease the cost and time of sequencing. Amplicons based next generation sequencing represents an attractive detection method. However, the new technologies have brought new challenges. In this presentation, consider the principle behind NGS technologies, as well interpretation of data. Furthermore, we consider some of the main clinical applications of NGS, taking into consideration that there will be a growing progress in this field in the forthcoming future.

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SS-MolPath-0004

CURRENT APPROACHES TO THE LABORATORY DIAGNOSIS OF TUBERCULOSIS

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ABSTRACT
Conventional laboratory methods for diagnosing TB are laborious and time consuming. Molecular methods currently being used are sensitive, rapid, and accurate, and diagnose TB in timely manner. This talk will give an over view of newer techniques being introduced in routine TB diagnosis.

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SS-MolPath-0006

NEW DIAGNOSTICS AND TREATMENT FOR THALASSAEMIA

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ABSTRACT
β -thalassemia is an autosomal recessive disorder which results in the formation of abnormal hemoglobin due to a variety of different mutations found in the HBB gene. These mutations render patient's incapable of producing correct form of hemoglobin. To identify HBB gene mutations in β-thalassemic patients, not included in the common-mutation panel of ARMS PCR, by sequencing HBB coding, intronic and promoter will identify novel and rare mutations

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SS-MolPath-0007

IMMUNOMAGNETIC SEPARATION OF CELLS: APPLICATION IN PATHOBIOLOGY

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ABSTRACT
Immuno-magnetic cell separation is a vital application for various basic and biomedical procedures. Through this technique, pure population of target cells is isolated from a heterogeneous mixture based on antibody conjugated magnetic beads. Alternatively, it is also known as rare cell separation technique. Purified cells are cultured to get an insight of their cellular or molecular characters which are extremely useful in molecular genetics, cell biology, proteomics, diagnostics and stem cell therapies. Recently, the concept of liquid biopsy in the detection of circulating tumor cells (CTCs) is primarily based on immuno-magnetic separation technique. CTCs are separated based on epithelial cell adhesion molecule (EpCAM) followed by immune-staining/fluorescence activated cell sorting (FACS) analysis to confirm malignant cells. In case of prostate cancer, less than 50 CTCs are found in 7.5 mL of the blood sample. These rare cells (CTCs) are captures by anti-beads which pass through a magnetic column placed in an external magnetic field of 0.5-0.7 T. Immuno-magnetically captured rare cells are subjected to a variety readout systems. Similarly, circulating endothelial cells (CECs) are getting importance of being an indicator of cardiovascular or endothelial injury in diagnostics. CECs, as rare cells, are captured through immune-magnetic separation system and subjected to molecular characterization. Despite clinical or diagnostic application, Immuno-magnetic separation technique is widely used in basic life science research including microbiology, water environmental sample, membrane potential studies and cell cycle studies. Purified population of target cells as a toll of specificity for the downstream applications is fairly possible through immune-magnetic separation technique.

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SS-MolPath-0008

ECONOMIZING MOLECULAR DIAGNOSTIC TESTING

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ABSTRACT
There is intense and growing interest in personalized medicine which is the practice of using genetic profiling to tailor medical care to individual's needs. Testing and treatment options are increasing with the advancement in field of medicine. Molecular diagnostic tests have very

high costs and parallel running conventional techniques further enhance the cost. The appropriate use of diagnostic testing and treatment so that opportunities for improved survival and quality of life are not missed is the goal of value-based approach. This presentation mainly emphasizes on the need for thorough evaluation before making decision on what makes a test "worth" the cost. Elimination of duplicative and unnecessary tests by adherence to evidence-based clinical pathways can result in cost savings that can then be invested in the appropriate use of innovative tests or treatments.

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SS-MolPath-0009

DNA SEQUENCE VARIANT CLASSIFICATION AND REPORTING

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ABSTRACT

With the advent of next-generation sequencing, genomics has rapidly moved from bench to bed side. Clinical laboratories are now performing genetic testing spanning genotyping, single genes, gene panels, exomes, genomes, transcriptomes, and epigenetic assays for genetic disorders. This advancement is coupled with new challenges in sequence interpretation. There is an ever-increasing detection of novel sequence variants in the course of testing patient specimens for a rapidly increasing number of genes associated with genetic disorders. While some phenotypes are associated with a single gene, many are associated with multiple genes. Our understanding of the clinical significance of any given sequence variant ranges from those in which the variant is almost certainly pathogenic for a disorder to those that are almost certainly benign. The challenge is the grey area flanked by these two certainties Given the complexity of the problem, an integrated approach is required to make the careful assessment before deciding the clinical significance of variants.

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SS-MolPath-0010

IMPORTANCE OF MOLECULAR TESTING IN INHERITED METABOLIC DISORDERS

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ABSTRACT

Previously Inherited Metabolic Disorders (IMDs) were defined as inherited disorders of the biosynthesis or breakdown of substances within specific pathways that were recognized by specific biochemical tests. In the genomic era many new IMDs are discovered, which do not fulfill the classical definition of IMDs. The new proposed definition of IMDs in the genomic era is "IMD should be defined to include any condition in which the impairment of a biochemical pathway is intrinsic to the pathophysiology of the disease". Targeted gene testing through Sanger Sequencing, massive molecular testing for several genes through Next-generation Sequencing and the Whole Exome/Genome sequencing has not only led to easier non-invasive testing for many IMDs, allowed better understanding of genotype-phenotype correlation for common IMDs but also expanded the spectrum of IMDs and led to discovery of many new IMDs.

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SS-MolPath-0011

NEXUS BETWEEN PERSONAL GENOMICS & PERSONALIZED MEDICINE

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ABSTRACT

The latest focus in human health research is to develop personalized medicines to cure a particular disease in a given patient. The most obvious causes of hereditary diseases are particular mutations in DNA sequences of a patient. The mutations (insertions, deletions, single nucleotide polymorphisms, copy number variations, etc.) could be in coding sequences (exons of the genes), or non-coding sequences (introns, or other regulatory sequences). The causes may also be epigenetic in some cases, affecting gene expression rather than mutation in DNA sequence. Next generation sequencing technologies offer massively parallel sequencing of large genomic parts, even whole genomes in a single reaction, producing huge amount of sequence data. These technologies enable genome-wide screening / analyses of the causative mutations within a few weeks' time. In this talk, I will particularly give a brief introduction of Illumina NGS platform, and its applications for whole genome sequencing, whole exome sequencing, and customized cancer panels. These are the most popular techniques among our customers (researchers as well as direct patients) for identifying genetic causes of the diseases. Personal genomics has a tremendous potential for selecting personal medicine.

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SS-MolPath-0012

THE PROMISE OF MOLECULAR PATHOLOGY: PROS AND CONS OF VARIOUS GENOME EDITING TECHNIQUES FOR CLINICAL APPLICATION

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ABSTRACT

Traditional healthcare system is at the doorstep for entering into arena of molecular medicine. The enormous knowledge and ongoing research has now been able to demonstrate methodologies which can alter the DNA coding. The techniques used to edit used to edit or change genome evolved from the earlier attempt like nuclease technologies, homing endonuclease an certain chemical methods. With ever monitoring evolution newer molecular techniques enter markets like the use of maganulease. Transcription activator like effector nucleases (TALENS), the use of Zinc-Finger Nucleases (ZFNs) which though reman in utility in various labs across the globe for quite some time now. These initial technologies suffer from lower specificity due to their off targets side effects. Moreover, from biotechnology's perspective the main obstacle was to develop simple but effective delivery methods into the host cells. With the discovery miRNA, tools like RNA interference were heavily relied upon as genome editing techniques with main used being to stop mRNA form translating into protein. These small RNAs including miRNa and siRNA have been widely adopted in the research laboratories to replace lab animals and cell lines. The latest discovery of CRISOR/ Cas9 technology seems more encouraging by providing better efficiency, feasibility and multi-role clinical application. This later biotechnological seems to take genome engineering techniques to the next of molecular engineering. This review generally discusses the various gene editing technologies in terms of the mechanism of action, advantages and side effects of various gene editing technologies.

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SS-MolPath-0013

BRCA1 PROMOTER GENE METHYLATION

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ABSTRACT

The triple-negative breast cancer (TNBC) subtype occurs in 18–30% of cases in Pakistan and is associated with younger age of onset, rapid growth, early metastasis and a worse prognosis than other breast cancer subtypes. Sporadic triple-negative tumors often show the same histological characteristics and clinical outcome as BRCA1-mutation carriers, several studies postulated that BRCA1 inactivation might also have a role in sporadic TNBCs. BRCAness is the phenotype that some sporadic tumors share with BRCA-mutated cancers. These tumors can have BRCA1 promoter methylation, a somatic mutation or another alteration causing a dysfunctioning BRCA pathway. A test for BRCA 1 promoter methylation as a sign of BRCAness may provide an indication for treatment with poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitors. BRCA1 CpG island promoter hypermethylation-associated silencing also predicts enhanced sensitivity to platinum-derived drugs to the same extent as BRCA 1 mutations. Most importantly, BRCA 1 hypermethylation has bene shown to be a predictor of longer time to relapse and improved overall survival in ovarian cancer patients undergoing chemotherapy with cisplatin.

In a resource limited setting it would be advantageous to screen for BRCA1 promoter methylation as it would enable better management of patients, through targeted therapy. Surrogates of methylation such as immunohistochemical staining with BRCA1 mAb could provide a more cost-effective testing method. To explore the utility of BRCA1 promoter methylation screening for breast tumors in Pakistan we investigated the frequency of BRCA 1 promoter methylation in a small cohort of patients.

The results from the study provide valuable information regarding the role of epigenetic modifications and BRCA 1 protein expression in breast cancer from a cohort of Pakistani patients.

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SS-MolPath-0014

BRCA1/2 GENE MUTATION SCREENING FOR THE HEREDITARY BREAST AND/OR OVARIAN CANCER SYNDROME IN BREAST CANCER CASES: UTILITY OF HIGH-RESOLUTION DNA MELTING (HRM) ANALYSIS

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ABSTRACT

Objective: To evaluate the utility of HRM-PCR as primary screening tool for the quick and low-cost variant detection in BRCA1/BRCA2 gene

Study Design: Prospective

Material and Methods: Ninety-eight unrelated diagnosed breast cancer patients from Breast Clinics, Liaquat National Hospital and Medical College Karachi, Pakistan, were included in this study. Genomic DNA was extracted using QIAGEN DNA mini Blood Kit Kit from venous blood. All

exons of BRCA1 and BRCA2 gene were amplified. Melting curve was analyzed by Rotor Gene software. Aberrant patterns were sequenced using Sanger sequencing method on GeXP genome Lab Genetic Analyzer. The sequence analysis was compared to the reference NCBI, BRCA1: NG_005905.2, NM_007294.3 (LRG_292) AND NCBI, BRCA2: NG_012772.3, NM_000059.3 (LRG_293). Variant Name was generated using online software Mutalyzer. Variants were searched in ClinVar Database for detailed information, including variant frequency and impact of change on protein. Variants found were confirmed by exploring BRCA Exchange database.

Result: This study identified 4 variants in the BRCA1 gene, (c.1067A>G, c.2612C>T, c.3113A>G, c.3548 A>G), and 3 variants in the BRCA2 gene, (c.865A>C, c.2971A>G, c.1114A>C). All variants tend to be polymorphisms. No known pathogenic mutation was found.

Conclusion: Our Study was able to find variants in our breast cancer patients. However, All variants were polymorphisms

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SS-MolPath-0015

GENOTYPE-PHENOTYPE CORRELATION AND HETEROGENEITY IN HEMATOLOGICAL DISORDERS

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MOLECULAR PATHOLOGY FREE PAPER ABSTRACTS

FP-MolPath-0001

USING MACHINE BASED LEARNING TOOLS FOR DECIPHERING ANTIMICROBIAL RESISTANCE EMPLOYING WHOLE GENOME SEQUENCING

Abdul Ahad, Muhammad Ramzan Khan, Aamer Ikram and Muhammad Salman
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ABSTRACT

Emergence of antimicrobial resistance (AMR) is a global health concern. AMR surveillance is a critical step within risk assessment schemes, as it is the basis for informing global strategies, monitoring the effectiveness of public health interventions. For outbreak and transmission investigation traditional culture- based and current molecular diagnostics methods can provide limited information that is often not sufficient. Nowadays large scale epidemiological, clinical and public health microbiology laboratories are increasingly adopting next generation sequencing (NGS) technologies, in their workflows. We review aspects and considerations for applying NGS and Machine learning approaches in the clinical microbiology settings, and highlight the impact of such implementation on the analytical and post-analytical stages of diagnosis. We discuss major applications, which include: (i) Target DNA and antigens identification; (ii) Molecular typing of epidemiological stains to monitoring pathogen during outbreaks; (iii) Investigation of genomic feature properties, such as the presence of antimicrobial resistance, virulence factors and comparative genome analysis; (IV) Use of Bioinformatics tools, Machine learning and Deep learning approaches to predict Antimicrobial resistance, Pathogenicity islands in bacterial genome and Minimum inhibitory concentrations. Typically, WGS analysis determinants previously identified AMR and existing information on biological mechanisms. To overcome this limitation, now machine learning models use to predict novel antimicrobial resistance. Being a high-resolution tool, high-throughput sequencing and machine learning approach will increasingly influence diagnostics, epidemiology, risk management, and real-time patient management and the prediction of antimicrobial resistance.

MolPath-0002

TOLL LIKE RECEPTOR-4 POLYMORPHISM AND GENE EXPRESSION OF IFN-β AND TLR4 IN HEPATITIS C PATIENTS

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ABSTRACT

Objective: Toll like receptors (TLRs) recognizes and binds particular molecular patterns of pathogens. HCV alters expression of TLR-4 in hepatitis C infection. In viral infections innate immune mechanism is the first line of defense. Type 1 interferon (IFN α, IFN β) is the key cytokines that are responsible for viral clearance in cells. Objective of this study is to analyze genetic variation of TLR 4 and expression of TLR4 and IFN-β in hepatitis C patients as compared to healthy controls

Study design: It was a comparative cross-sectional study.

Material and Methods: Eighty-Seven subjects were recruited for each of the two study groups. Five ml of EDTA blood from patients of HCV and healthy controls was collected and processed. This study has analyzed the single gene polymorphism (SNP) of TLR4 (9rs4986791) by PCR- RFLP and also analyzed relative gene expressions of TLR4 and IFN-β in hepatitis C patients as compared to healthy controls by real time PCR technique.

Results: Expression of both genes TLR 4 and IFN-β was down regulated in hepatitis C patients. Polymorphisim analysis revealed that Allele T was more frequent in HCV positive cases as compared to healthy controls thus suggesting T allele as high-risk allele in HCV patients. TLR4 gene polymorphism (rs4986791 A/T) decreased gene expression of IFN-β and TLR4 in hepatitis C patients.

Conclusion: This study concludes that TLR4 SNP C/T at rs 4986791 contributes in hepatitis C disease development as well as down regulation of genetic expression of TLR4 and IFN-β.

Keywords: TLR 4 Interferon- β, HCV, Single nucleotide Polymorphism

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FP-MolPath-0003

DIAGNOSIS OF HUMAN PAPILLOMA VIRUS IN THE SCREENING FOR CERVICAL CANCER: A PILOT STUDY FROM KARACHI

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ABSTRACT

Objective: Persistent infection with high-risk human papillomaviruses (HPV) is determined as a risk factor for developing cervical cancer. Following infection of epithelial cells, the HPV DNA becomes established in the epithelium and can cause cervical intraepithelial neoplasia (CIN). Screening for cervical cancer using Pap smear identifies neoplastic changes that have already occurred. We investigated how HPV DNA based testing can be used to identify early CIN lesions for improved prognosis of cervical cancer.

Study design: Retrospective review of specimens received for testing by High Risk HPV AKUH laboratory between Jan 2017 and June 2018.

Materials and Method: Cervical specimens were tested by High Risk HPV DNA Hybrid Capture 2 method (Digenene USA) at the AKUH laboratory. Results were correlated with Pap smear and biopsy results where these were available.

Results: A total of 182 cervical were received. Of these 22 were found to be positive for High Risk HPV DNA. Of those positive, age of patients was 41 y (24-62y). Pap smears were available in 12 cases of which 3 were positive for LSIL/ squamous cell carcinoma. Histopathology was available for 11 cases of which 10 were positive (three were CIN I, six were CIN III) for carcinoma.

Conclusion: Our results revealed that the majority of cases diagnosed with HPV were coincident with advanced squamous cell carcinoma, CIN III lesions, indicating a delayed diagnosis of the infection. HPV DNA diagnosis is an effective way of diagnosing early CIN lesions and should be used for early screening and identification of cervical cancer. This will allow early and improved interventions for the disease.

Key Words: Cervical intraepithelial neoplasia, Low grade squamous cell intraepithelial lesion, High grade intraepithelial lesion.

FP-MolPath-0004

CYTOGENETIC ANALYSIS OF PATIENTS WITH AMBIGUOUS GENITALIA

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ABSTRACT

Objective: To determine the Karyotype of patients with ambiguous genitalia for gender assignment.

Study design: Cross sectional study.

Place and duration of study: Department of Haematology, Armed Forces Institute of Pathology (AFIP) RWP from August 2017 to August 2018.

Patients and methods: All patients with ambiguous genitalia referred to AFIP for Cytogenetic analysis/Karyotyping were included in this study. Patients referred for karyotyping due to other indications, e.g. Primary Amenorrhea, were not included. Cytogenetic analysis was done by using Conventional Giemsa banding. Karyotypes were interpreted using the International System for Human Cytogenetic Nomenclature (ISCN).

Results: Out of total 60 patients with genital ambiguity, all (100%) yielded successful cytogenetic culture. 39 cases (65%) had a 46, XY karyotype while 20 cases (33.3 %) had a 46, XX karyotype. 01 case (1.67%) yielded a 45,X/46,XY karyotype (45,X/46,XY Mosaicism). 42 cases (70%) had a positive consanguinity. 09 cases (15%) who were considered to be females were found to be genetic males (46,XY) while 06 cases (10%) who were considered to be males turned out to be genetic females (46,XX). 01 case (1.67%) who was being raised as a female was found to be 45, X/46, XY mosaic.

Conclusion: Cytogenetic analysis is essential to unfold the genotype and help in assignment of gender in patients with ambiguous genitalia.

Key Words: Ambiguous genitalia, karyotype, cytogenetic analysis.

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FP-MolPath-0005

INTEGRATIVE GENOMICS AND GENE ONTOLOGY MAY HELP IN THE IDENTIFICATION OF NOVEL BIOLOGICAL PATHWAYS IN PATHOGENESIS OF CAD

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ABSTRACT

Introduction: Integrative genomics and gene ontology may help in the identification of novel biological pathways in the pathogenesis of CAD.

Objectives: Objective of the study was to identify the direct and indirect protein interactions of the 13 CAD risk genes and the 5 cytokine genes in PCAD.

Study Design: Case-control study.

Methodology: The study was conducted at Army Medical College, in association with University College London (UCL), London, United Kingdom (UK). 340 PCAD patients and 310 age and sex matched controls were recruited. Serum IL18, TNFA, IL6 and IL10 levels were measured using ELISA (Invitrogen). The SNPs were genotyped using TAQMAN and KASPar assays. Data analysis was done using standard SPSS software version-21 (SPSS Inc, Chicago, Illinois, USA). The protein-protein interaction (PPI) network was generated using *STRING* version 9.0, Genemania and I-Tessar web.

Results: The patients of PCAD had mean ± SD age of 42 ± 3.80 years consisting of 329 males and 11 females. IL-6 works in close interaction with IL-6R, STAT3 and NFKB1. While MRAS, MIA3 and SORT1 interact with each other CXCL-12 mediates its actions by interacting with IL-18, JAK-2 and CCR4. LPA interacts closely with APOB and LPL acts via interaction with APOA4 and APOA5.

Conclusion: The correlation between gene risk scores and serum cytokine levels can aid in the analysis of complex networks to understand the pathogenesis of PCAD. Gene Ontology may be the way forward to unravel the pathogenic mechanisms in non-communicable diseases like PCAD.

Key Words: Interplay; Immune inflammatory pathway; Gene score; Cytokine levels

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FP-MolPath-0006

ROLE OF THROMBOXANE A2 IN FIRE BURN PATIENTS

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ABSTRACT

Objective: The objective of this study was to determine whether there is an association between TXA2 gene and fire burn patient.

Design and Patients: 50 Burn patients were enrolled for this study as they were admitted due to their burn

condition, having fire or scald burn, admission within 24 hours after injury were included in this study.

Methodology: For mutation detection whole blood was collected in Acid Citrate Dextrose tube one time only when patient admitted. DNA extraction was performed using Qiagen spin column kit according to manufacturer's protocol. PCR was performed with primer designed for TXA2 gene.

Results: Out of 50 patients, 30 were female and 20 were male age ranging between 14-50 years with more than 20% TBSA. TXA2 gene polymorphism were genotyped which showed significant association between TXA2 and fire burn patients.

Conclusions: Thromboxane A2 (TXA2), predominant product of cyclooxygenase, is produced from arachidonic acid in human platelets. TXA2 is extremely labile substances, unstable in aqueous solution, since it is hydrolyzed within about 30 seconds to TXB2. Due to mutation, there is continuously aggregation of platelets cause ischemia formation at the side of injury which results amputation.

Keywords: ThrmoboxaneA2, Fire burn, Ischemia, Amputation,

FP-MolPath-0007

EDAR MUTATION IN FAMILIAL TOOTH AGENESIS INVOLVING INCISORS AND CANINES

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ABSTRACT

Background: Tooth agenesis is the most prevalent craniofacial congenital malformation and characterized by the absence of variable number of permanent teeth, most commonly premolars in both upper and lower jaws. It may be inherited in autosomal dominant, autosomal recessive or X-linked fashion. We report on nine affected members of a Pakistani family afflicted with autosomal dominant nonsyndromic tooth agenesis.

Material and Methods: Clinical information on members of the family was collected. Mutation was identified through linkage analysis using high-density SNP genotypes coupled with exome sequencing,

Result: The malformation is bilateral in the majority of cases, and hallmark feature is the absence of lateral incisors, canines and central incisors, and premolars are involved less often. Predominantly maxilla is involved. Affected individuals also have some other features such as diastema between central incisors, well-developed frenum, peg-shaped lower incisors and delayed exfoliation. We identified a novel nonsense mutation in *EDAR* (c.1302G>A, p.(Trp434*)). The variant is deduced to create a premature termination codon that leads to the truncation of the protein and thus the deletion of 15 native residues. Heterozygous *EDAR* mutations most commonly cause hypohydrotic ectodermal dysplasia, but recently one nonsense and eight missense mutations have been reported in nonsyndromic tooth agenesis. The phenotype of the large family we present is highly variable and different from the reported EDAR-related nonsyndromic tooth agenesis in which third molars and second premolars are affected mostly. Affected individuals exhibited also other dental anomalies such as diastema, prominent labial frenum, delayed exfoliation, over-erupted upper incisors, peg-shaped lower incisors and malocclusion; most of these features were not reported in any of the reported *EDAR*-related tooth agenesis. Notably, there was no conspicuous involvement of other ectodermal appendages.

Conclusion: Our findings add to the wide variability of the manifestations of the EDAR deficit and indicate that *EDAR* should be included in the list of gene panels used for screening tooth agenesis.

Key words: oligodontia, hypodontia, diastema, Pakistani family

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FP-MolPath-0008

ROLE OF STR ANALYSIS FOR EXCLUDING MATERNAL CELL CONTAMINATION IN CVS SAMPLES

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FP-MolPath-0009

MOLECULAR SURVEILLANCE OF DRUG RESISTANCE IN PLASMODIUM FALCIPARUM MALARIA USING TARGETED AMPLICON DEEP SEQUENCING (NGS)

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FP-MolPath-0010

DIAGNOSIS OF MUSCULAR DYSTROPHIES USING MULTIPLE LIGATION PROBE-BASED AMPLIFICATION (MLPA) BASED ASSAYS

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MOLECULAR PATHOLOGY POSTER PRESENTATION ABSTRACTS

P-MolPath-0001

GENETIC AND BIOCHEMICAL PROFILING OF PALB2 WITHIN PALB2-BRCA2 INTERFACE IN PAKISTANI BREAST CANCER PATIENTS

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ABSTRACT

Breast cancer is one of the leading reasons of deaths among women in the world. One of the major causes of breast cancer is ionizing radiations that create DNA double strand breaks. Nature has blessed cells with multiple repair pathways of which homology dependent DNA repair is of central importance. This utilizes recombinase for the homology search and repair. In this repair system, the association of BRCA2 (breast cancer susceptibility type 2) and PALB2 (partner and localizer of BRCA2) is crucial whose disruption can predispose to breast/ovarian syndrome. PALB2 is the successor of BRCA2 in cancer prevention and has been identified to show subtle point mutations in different population-based studies. We carried out our studies to investigate the Pakistani population for the identification of possible point mutations in PALB2 within the domain that interacts BRCA2. High quality DNA was isolated from both healthy and tumorous samples which was then employed in PCR based amplification using gene specific primers. Gel extraction method was utilized for the isolation of the PCR amplified gene products from the agarose gels which were then sent for DNA sequencing whose results would be crucial in better understanding of the mutational status of PALB2 in Pakistan. Method for protein isolation from tissue samples was also optimized to be used in Western blotting-based protein expression analysis. Moreover, the microscopic slides for histopathological studies confirmed the progression in tumourous cells only. Our studies on PALB2 would help Pakistani population for predisposition of breast cancer.

Key Words: PALB2, BRCA2, Homologous Recombination Repair Pathway

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P-MolPath-0002

EXAMINING THE IMPACT OF DELAYED TRANSPORTATION OF CYTOGENETIC SPECIMENS ON CELL CULTURE FAILURE RATE

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ABSTRACT

Objective: Cytogenetics is a field of study that deals with chromosomal abnormalities which are common cause of developmental delay, congenital malfor-mations, mental retardations and infertility. Cytogenetic analysis is also significant for the diagnosis and management of oncologic and hematologic disorders. The purpose of this study was to evaluate how delayed transportation of blood and amniotic fluid specimen influence rate of cell culture failure.

Materials and Methods: There are many possible origins of culture failures. It can be the result of improper specimen collection and laboratory technique, inadequate sample quantity, collection under nonsterile conditions and delay in transport. All these parameters were thoroughly investigated for the analysis of specimen collected from January 2016 to December 2017 using Six Sigma tools. Importance of education and training were emphasized during the study to the staff and faculty dealing both in and out-patients.

Results: Out of 1854 blood specimen collected in 2016, 48 resulted in cells culture failure due to delayed transportation of approximately 3 to 4 days from different collection points outside and within Karachi. Overall, cell culture failure rate was 2.7% for the year 2016. In 2017 several lecture sessions were conducted which helped in reducing failure rate to 1.37% (25/1835).

Conclusions: On comparing the results of both the years, delay in specimen transportation was concluded as a considerable factor in case of culture failure, which directly lead to the increase in overall laboratory cost. It can be improved by educating the respective staff through lectures and regular distribution of specimen collection and transportation guidelines.

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P-MolPath-0003

SCREENING OF B-GLOBIN GENE (*HBB*) FOR RARE MUTATIONS IN B-THALASSEMIA PATIENTS USING SANGER SEQUENCING

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ABSTRACT

Objective: β -thalassemia is an autosomal recessive disorder which results in the formation of abnormal hemoglobin due to a variety of different mutations found in the *HBB* gene. These mutations render patients incapable of producing correct form of hemoglobin. The aim of this study was to identify *HBB* gene mutations in β-thalassemic patients, not included in the common-mutation panel of ARMS PCR, by sequencing *HBB* coding, intronic and promoter.

Materials and Method: A total of 10 samples previously tested for *HBB* gene mutations by ARMS PCR common-panel (i.e. IVS 1-1, IVS 1-5, Codon 8/9, Codon 41/42 and 619bp deletion) were analyzed by Sanger sequencing. Two

healthy subjects were included as negative controls. Genomic DNA was isolated and *HBB* gene was amplified. Column purified amplified products were utilized for bidirectional cycle sequencing (Big Dye Terminator, ABI, USA).

Results: In the present study, a total of 10 samples were analyzed. Four were males and six females. The Mean of the patients was three years. All patients were diagnosed as β-thalassemia major based on their family history, clinical and laboratory findings. On average, patients were receiving transfusions every 2nd week. Seven rare mutations in *HBB* gene were detected including point mutations. The mutations spanned in the promoter region HBB:c.138C>A (-88 C>A), exon1 HBB:c.17_18 delCT (Codon5 –CT), HBB:c.47G>A (Codon15 G>A), HBB:c.92G>C (Codon30 G>C), HBB:c.50A>C (CAP+1 A>C), exon2 HBB:c.118C>T (Codon39), and intron2 HBB:c.315+1G>A (IVS II-I G>A). All control subjects showed normal *HBB* gene sequence. In addition, a polymorphism T>C in codon3 at position HBB:c.59 was detected in majority of the patients and controls.

Conclusion: Although ARMS PCR is a fast and convenient method for detection of common mutations in the *HBB* gene, a small subset of patients may be missed because of rare mutations, which would require other means for diagnosis. Sanger sequencing is an accurate and robust technique to manage such patients.

Key Words: Thalassemia, β-globin, Sequencing.

P-MolPath-0004

DIAGNOSIS OF ALPHA-GLOBIN GENE (*HBA1 AND HBA2*) FOR RARE MUTATIONS IN ALPHA-THALASSEMIA PATIENTS USING MLPA

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ABSTRACT

Objective: The goal of this study was to identify HBA1 and HBA2 gene mutations in Alpha thalassemia patients.

Materials and Method: Genomic DNA was isolated and HBA1 and HBA2 gene were amplified by Multiplex Ligation-dependent Probe Amplification (MLPA), detected by Capillary electrophoresis and analyzed by Coffalyser software. This method is relatively simple and robust multiplex PCR method for detecting chromosomal DNA copy number changes in multiple targets. (MRC Holland)

Results: In this study, a total number of 36 samples were analyzed from May 2017 until Sep 2018. Of these 28 were males and 08 were females and nine had a history of blood transfusion. Four were patients were previously diagnosed as Beta-thalassemia major In total, 10 cases of alpha thalassemia were diagnosed. Eight were diagnosed with Alpha thalassemia 3.7 Deletion, one with Alpha thalassemia trait and one with a compound mutation detected as HS40 deletion. Results were compared with patient family history, hematological parameters and other clinical and laboratory findings.

Conclusion: The molecular testing of α-thalassemia has been challenging due to the complexity of the globin gene mutations. MLPA is a convenient choice for diagnosing deletions and can assist in the identification of alpha-thalassemia.

Key Words: Multiplex ligation dependent probe amplification, Hemoglobin Alpha,

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P-MolPath-0005

MOLECULAR CHARACTERIZATION & PREVALENCE OF ACTIVE HEPATITIS DELTA VIRUS INFECTION IN HBSAG POSITIVE PATIENTS IN PAKISTAN

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ABSTRACT

Objective: This study was conducted to determine the prevalence & molecular characterization of active HDV infection in HBsAg positive patients from all four provinces of Pakistan.

Methodology: 190 HBsAg positive serum samples were collected from all four provinces of Pakistan; KP, Baluchistan, Punjab and Sindh. ELISA confirmed samples were subjected to real-time PCR detection of viral RNA. Further, genotyping of HDV was performed by using PCR-RFLP method while mutation analysis and molecular phylogeny was reconstructed by sequencing the partial fragment of HDAg coding gene.

Results & conclusion: The results demonstrated high infection rate of HDV; 24.7% in Pakistani population where male gender and age group ranging from 21 to 40 years had comparatively higher infection rate. Our analysis shows that only one genotype of this virus (HDV-I), is associated with active infection of HDV in the country. However genetic diversity of this genotype in Pakistani population indicates towards its multiple origins of spread.

Keywords: HDV, Phylogeny, Pakistan

P-MolPath-0006

MOLECULAR CHARACTERIZATION OF > ACNE VULGARIS IN PAKISTANI POPULATION

Sumaira Akram

ABSTRACT

Acne vulgaris is a chronic inflammatory disease of skin characterized by inflammed and non-inflammed lesions in the form of papule, pustule, nodule and cysts. Global incidence is 1 case per 947 individual per year. Prevalence of acne in Pakistan is 1 case per 633 individual per year. Inflammation plays a strong role in pathogenesis of acne and proinflammatory cytokines are key players in these inflammatory events. Single nucleotide polymorphism in

inflammatory cytokine like TNF alpha are considered to influence the pathogenesis of acne. The current case control study was aimed to evaluate the potential role of TNF-alpha-863 (rs1800630) and -1031 (rs 1799964) in development of acne vulgaris in Pakistani population. Blood samples of 100 acne patients and 100 aged and gender matched healthy control were recruited from same ethnic group. Serum TNF and CRP levels were measured by Enzyme -linked Immunosorbent assay. TNF-alpha genotyping was performed by polymerase chain reaction (PCR) and restriction fregment length polymorphism (RFLP). Elevated TNF-alpha were observed in acne patients vs. unaffected controls (P = 0.001). CRP levels were also high in patients compared to controls (P < 0.001). The genotypes of TNF-alpha -1031T>C showed marginal association when compared to the controls ($\chi 2$= 5.71, P = 0.05). The frequency of heterozygous TC genotype in patients were high than in controls. However, no

significant association was found between the variant allele C and the disease. Moreover, Significant association between TNF-alph-863 C>A polymorph-hism and acne vulgaris has been observed. The variant genotype CA+AA was more prevalent among the acne patients (P = 0.02, $\chi 2$ = 4.62). Higher incidence of -863 A allele and CA+AA genotype was found in acne patients compared to healthy control indicating the increased risk of acne patients (OR = 0.5, 95%CI = 0.304-0.909, P = 0.02). Significant association of disease severity with TNF-alpha and CRP was found in patients (P = 0.001). Non-significant relationship of variant genotype at TNF-alpha-1031 and -863 and serum TNF-alpha and CRP levels were found in patients. It can be concluded that TNF-alpha rs1800630 might play a key role in acne formation in studied population.

Keyword: TNF, RFLP, ACNE VULGARIS.

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P-MolPath-0007

PREVALENCE OF CHROMOSOME ANEUPLOIDIES IN PAKISTAN

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ABSTRACT

Background: Cytogenetic analysis is a valuable investigation for the diagnostic work up of suspected aneuploidy. The objective of this study was to determine the frequency of aneuploidies reported in the Pakistani population. The most common cause of chromosome aneuploidies is nondisjunction, which can occur during meiosis or during the early stages of post zygotic development.

Materials and Method: 72 hours PHA stimulated lymphocyte cultured Cytogenetic results of 2025 Individuals, reported between January 2016 and August 2018, were reviewed retrospectively. All individuals were suspected for aneuploidies karyotyping analysis was performed in the cytogenetic laboratory of Molecular Pathology Section, The Aga Khan University Hospital.

Results: A total of 2025 individuals were karyotyped. Abnormal karyotypes were found in 886 (43.75%) patients. 1266 individuals (62%) reported with history of suspected Down syndrome, out of those 775(61%) having (mean age 7 months), 464 males and 311 females were reported positive for trisomy 21. 665 (32.83%) females, (mean age 15.3 years) came with history of Turner syndrome, out of those 90 cases (13.53%) reported to have a monosomy X. Out of 68 individuals, (mean age 24 Years) with suspected history of Klinefelter syndrome 14 (20.58%) cases reported positive for Klinefelter syndrome. Out of 14 individuals, (mean age 1 month) with suspected history of Edward syndrome 4 cases (28.57%) 04 females were reported with trisomy 18. Out of 13 individuals, (mean age 1 month) with suspected history of Patau syndrome 3 cases (23%) 2 females and 1 male were reported with trisomy 13. Furthermore, Down syndrome was the commonest aneuploidy identified.

Conclusions: In comparison to the regional data, the prevalence of chromosome aneuploidies in the Pakistani population undergoing cytogenetic analysis was relatively higher.

Keywords: Aneuploidy, Chromosome aberrations, Cytogenetic analysis, Karyotypes.

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P-MolPath-0008

HBV/HDV CO-INFECTION IN ADULTS–SIX YEARS OF MOLECULAR DIAGNOSTIC EXPERIENCE AT LIAQUAT NATIONAL HOSPITAL AND MEDICAL COLLEGE KARACHI

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P-MolPath-0009

BIOCHEMISTRY & AMP; ENVIRONMENTAL RISK FACTORS ANALYSIS FOR PEDIATRIC ALL: A PROSPECTIVE STUDY OF CHILDREN HOSPITAL LAHORE, PAKISTAN

Nasir Mahmood

P-MolPath-0010

IMPLICATION OF IL-10 AND IL-28B GENE POLYMORPHISM WITH SUCCESSFUL ANTI-HCV THERAPY AND VIRAL CLEARANCE

Gahzala Rubi, Ali Javed, Sehr Maha

HAEMATOLOGY SCIENTIFIC SESSION ABSTRACTS

SS-Haem-0001

IMPORTANCE OF GENETIC TESTING IN THALASSAEMIA & ITS CLINICAL APPLICATION

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ABSTRACT

Background: Differentiation between thalassaemia major and thalassaemia intermedia at presentation is not uniformly characterized, for which an absolute criterion needs to be developed. This study investigated the primary and secondary genetic modifiers to develop a laboratory finding by forming different genetic mutational combinations seen among thalassaemia intermedia patients and comparing them with thalassaemia major.

Material and Methods: This cross-sectional study analyzed 315 thalassaemia intermedia patients. 105 thalassaemia major patients were recruited on the basis of documented evidence of diagnosis and were receiving blood transfusion therapy regularly. Various mutational combinations were identified and comparison was done between thalassemia intermedia and major using statistical software STATA 11.1.

Results: The mean age of the total population was 5.9 +/- 5.32 years of which 165 (52%) were males. Of the two groups (thalassemia intermedia and thalassemia major), IVSI-5, IVSI-1 and Fr 8-9 are more prevalent among the thalassemia intermedia cohort. When comparison was done between the thalassemia intermedia and thalassemia major patients, it showed significant results for the presence of Xmn-1 polymorphism.

Conclusion: The presence of IVSI-5 homozygous with Xmn-1, IVSI-5 heterozygous with Xmn-1, Cd 30 homozygous with or without Xmn-1 and IVSI-1 homozygous or heterozygous either with or without Xmn-1 proved to be strong indicators towards diagnosis of thalassemia intermedia

Recombinant TSH is effective in providing exogenous TSH stimulation for patients with differentiated thyroid cancer on thyroid hormone-suppressive therapy. It allows for detection of thyroid remnant and metastases by radioiodine scan and by serum thyroglobulin determination. The sensitivity and image quality of the WBS are similar after rTSH and after THSH withdrawal in the majority of patients. The equivalent 100% sensitivity of rTSH- and withdrawal-stimulated serum thyroglobulin measurement alone in identifying patients with radioiodine uptake outside the thyroid bed may eventually lead to more extensive use of serum thyroglobulin testing after rTSH, with more selective application of radioiodine whole body scan.

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SS-Haem-0002

BETA THALASSEMIA – AN INSIGHT INTO THE OMICS OF BETA GLOBIN GENE DISORDER

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ABSTRACT

Symptoms of beta-thalalasemia are due to anemia and iron overload. Despite regular blood transfusion and iron chelation, delayed growth, risk of infection and thrombosis, asthenia and exercise intolerance remains problem. Use of Hydroxyurea (HU) has shown to decrease the need of blood transfusion in a subset of patients. One consistent finding is a subjective improvement in quality of life, vitality, better exercise tolerance, improved food intake and energy level despite of minimal or no change in hemoglobin level. In Pakistani population, most cases of IVS1-1, mutation in Codon 30, Cap+1, Hb-E and some cases of IVS1-5 and Fr 8-9 have consistently ameliorated the need of transfusion on HU. Genetic modifies like XMN-1 polymorphism, BCL11-A, and others have important bearings on producing γ-globin chains. 44% of β-thalalasemia major patients have XMN-1 polymorphism (homozygous or heterozygous). Its presence is a strong predictor for a response to hemoglobin F augmentation. On the other hand, BCL11A is down-regulated during hematopoietic cell differentiation. Proteomics, metabolomics and metallomics studies have found a difference in patients before and after exposure to HU. Transferrin receptor protein-1 is found to be down-regulated while hemopexin and haptoglobin unregulated. Alteration in fatty acid, glycolytic, galactose, pyruvate, propanoate, glycerophospholipid and sucrose metabolism along with fatty acid elongation in the mitochondria, pointing towards the shi9f of metabolism in beta-thalassemia patients in comparison to healthy individuals. Biometal dysregulation of at least 8 metals (vadium, chromium, iron, cobalt, Ni copper, rubidium and lead) have differential distribution when compared with HU untreated samples. This possibly translates into improved quality of life and exercise tolerance. Treatment with HU normalize and these omics to normal baseline as those of control population. This suggests that apart form gamma globin gene augmentation, HU has a far implication n cellular metabolism and energy production.

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SS-Haem-0003

BREAKTHROUGHS IN THE TREATMENT OF THALASSAEMIA MAJOR

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ABSTRACT

Beta thalassaemia is an inherited disorder leading to absent or reduced synthesis of beta globin chain of adult haemoglobin which result in ineffective erythropoiesis, chronic haemolysis and severe anaemia requiring blood transfusions. The availability of blood transfusion and iron chelation now allow long term survival of these patients. Haemopoietic stem cell transplant from HLA matched donors is potentially curative option for this disorder. However, it is a very expensive treatment modality and also associated with fatal complications. Gene therapy using autologous stem cell transduced ex vivo with a lenti viral vector among patients with thalassaemia major resulted in increased level of HbA, reduced transfusion requirements and even no transfusion requirements in some patients. The lenti globin vector is self-inactivating and contains large elements of Beta globin locus control region. Ineffective erythropoiesis is the key pathophysiological mechanism underlying severe anaemia in patients with Beta thalassaemia major. Amelioration of ineffective erythropoiesis and iron dysregulation in Beta thalassaemia major are driving research into novel therapeutic options. Of these Janus Kinase 2 (JAK-2) inhibitors, activin receptor-II ligands traps sotatercept, luspatercept, gene editing targeting transcription factor BCL II A which suppresses expression of gamma-globin and use of mini-hepcidin are at the most advanced stage of development

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SS-Haem-0004

DIAGNOSTIC MODALITIES FOR G6PD DEFICIENCY TESTING

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ABSTRACT

G6PD, an inherited condition, is predominantly seen in males. Many of these patients remain undiagnosed for years. Severity of haemolytic anaemia varies among individuals with G6PD deficiency, making diagnosis more challenging in some cases. Diagnostic modalities for G6PD deficiency include simple tests like peripheral film and Heinz body preparation, traditional tests like methaemoglobin reduction test as well as advanced tests like biosensor and quantitative Trinity Biotech. Recently, genotyping has proved useful in diagnosis. Identification of G6PD deficiency and patient education regarding medications and foods is critical to prevent future episodes of haemolysis. With the right care, G6PD deficiency should not keep a person from living a healthy active life.

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SS-Haem-0005

RATIONAL USE OF MOLECULAR MARKERS IN MYELOPROLIFERATIVE NEOPLASMS

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ABSTRACT

Molecular testing has become focus of clinicians and oncologists during the last one decade. Genetic analysis starting from cell cultures for chromosomal studies to present day sequencing has led to explosion of information whether relevant or irrelevant. The use of these techniques are now becoming part of protocols in diagnosis, targeted therapy, monitoring and prognostic scoring system of many cancers in general and haematological malignancies in particular e.g. in acute leukemias, myelodysplasia, myeloproliferative neoplasms and lymphoproliferative disorders.

The diagnostic importance of Philadelphia chromosome in Chronic Myeloid Leukemia (CML) is well known. Monitoring of CML patients on Tyrosine Kinase Inhibitors by molecular analysis of BCR-ABL1 has been done to see the depth of response to the therapy. On the other hand in Philadelphia negative MPNs, mutations in Janus Kinase2 (JAK2V617F) and Calreticulin are seen with frequency of 95% & 0% in Polycythemia Vera, 55% & 25% in Essential Thrombocythemia and 60% & 25% in Primary Myelofibrosis. JAK2 Exon 12 & MPL Exon 10 mutations are infrequent. Now with the availability of whole exome sequencing and detection of additional alterations in genes like TET2, DNMT3A, IDH1/2 ASXL1 etc. in cases of MPNs the picture is becoming more and more confusing, since demonstration of characteristic bone marrow morphology is still the requirement for the diagnosis of myeloproliferative neoplasms according to WHO classification of tumors of haematopoietic and lymphoid tissues (2017).

The availability and standardization of these tests in diagnostic centers and hospitals is still not at a desirable level in our country mainly due to lack of qualified experts in this field. Since these tests involve high cost and most of the time patients are required to bear the expense, it is therefore mandatory for clinicians to understand the clinical relevance and utility before advising these expensive tests. The genetic analysis should be advised only when clinical benefits outweigh the burden of cost. Injudicious use of these tests not only burdens the patients but also generates irrelevant data.

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SS-Haem-0006

PHILADELPHIA NEGATIVE MPN'S

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ABSTRACT

WHO 2016 has emphasized on the compliance of bone marrow morphology in diagnostic criteria of various MPNs. Morphology of megakaryocytes is particularly important at the time of diagnosis as it can help to differentiate between MF and ET. Role of BM examination at the presentation will be discussed in the talk. Various prognostic scoring systems will be discussed briefly

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SS-Haem-0007

ROLE OF TARGETED THERAPIES FOR MOLECULAR MONITORING IN CURRENT MANAGEMENT OF CML

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ABSTRACT

The molecular understanding of diseases has helped in fine tuning the diagnosis and treatment of many hematological disorders with significant improvement in long term survival of many malignant Hematological disorders. CML is an excellent example of this Up till 2002, Stem cell transplant has been the only cure in younger patients. Molecular diagnosis, MRD monitoring and emergence of Tyrosine Kinase inhibitors (TKI's) has revitalized the treatment of Chronic phase CML. 8 years follow up of patients on Imatinib (IRIS trial) revealed that estimated OS is 85% in patients who could continue the drug.

Second generation bcr-abl inhibitors, Nilotinib and Dasatinib, are much more effective. Randomized phase 3 studies of Nilotinib 300 mg BD (ENESTnd trial) and dasatinib 100 mg OD (DASISION trial), compared with Imatinib, have shown an MMR of 76, 73 and 46 % respectively. Cumulative Incidence of MR4.5 at 48 months is 40, 42 and 23 % respectively. Bosatinib is approved in cases resistant to above drugs & Ponatinib for those having T315I mutation.

STIM1,2 trial, EURO-SKI study and STOP 2G-TKI study have demonstrated that 40-50 % patients who have attained MR4-4.5 for 2-4 years before the drug is discontinued, maintained the remission during follow ups beyond 2 years. This is largely possible with the availability molecular monitoring (bcr-abl quantification by QT-PCR). The newer TKI, however, are quite costly (USD 30,000-40,000 / year). It appears that molecular medicine has revolutionized outcome of chronic phase CML patients who appear functionally cured with newer TKI,s with a likely life expectancy of that of general population

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SS-Haem-0008

SERIOUS COMPLICATIONS OF BONE MARROW TRANSPLANT

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ABSTRACT

Following haematopoietic stem cell transplantation, recipient will be immunocompromised and may also have treatment related organ and tissue damage. Therefore, transplant recipients require careful monitoring in the early post-transplant period to ensure that complications are recognized early, so that maximum treatment options can be available and may be more effective.

The early serious complications are oral mucositis, acute graft versus host disease (GVHD), graft failure, early infections and organ injury/toxicity. The organ injury and toxicity following transplant can include hepatic veno-occlusive disease (VOD), renal failure, pulmonary toxicity, thrombotic microangiopathy (TMA) and cardiovascular complications.

In our study of last three years bone marrow transplants, the major serious complications were of pulmonary origin. The majority not diagnosed ante mortem and was the most common cause of death in BMT recipient.

Conclusion: In our view due to inadequate diagnostic tools and under diagnosis of pulmonary complications our transplant recipient probably may not received appropriate therapy for potentially treatable pulmonary complications.

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SS-Haem-0009

IMPORTANCE OF MOLECULAR ANALYSIS IN RARE BLEEDING DISORDER PATIENTS

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ABSTRACT

Rare bleeding disorders (RBDs) include the inherited deficiencies of coagulation factors, usually transmitted as autosomal recessive disorders. As a consequence of the rarity of these deficiencies, the type and severity of

bleeding symptoms, underlying molecular defects and the actual management of bleeding episodes is not well established. We determined the molecular analysis of RBDs in our population. All RBDs patients enrolled at our institute were included. Blood samples were collected for complete blood count, coagulation assays and genetic characterization. Out of 550 patients diagnosed with inherited coagulation bleeding disorders, 115 subjects had RBDs (21%). Among them, 45% were male and 55% female. Median age of patients was 11 years (range: 12 days to 37 years). Moreover, history of consanguinity was present in 85% cases and significant family history of bleeding in 60% patients. The most common deficiency was FXIII and FVII (n=32, 27.8%) followed by fibrinogen deficiency (n=26, 22.8%). Genetic characterization was carried out in 74/115 (64%) patients with 19 novel mutations. The study shows that autosomal recessive bleeding disorders are common in Pakistan, with FXIII and FVII being the most common, and have severe bleeding phenotype. Thus it identifies that the underlying molecular defect not only facilitates a definitive diagnosis of bleeding disorders, but also helps with the clinical prognosis, and enables genetic counseling for the prevention of these disorders.

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SS-Haem-0010

RECENT ADVANCES IN HEMOPHILIA MANAGEMENT: POSSIBILITIES IN PAKISTAN

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ABSTRACT

Haemophilia is an inherited bleeding disorder in which there is deficiency of factor VIII and Factor IX. The affected gene is F8 and F9 present on X chromosome. This results in deep tissue bleeding such as intra-articular, intramuscular and intracranial bleeding. The most recent advances in haemophilia are gene therapy and point-of-care joint ultrasound.

The most common clinical presentation is joint pain and bleeding. Repeated intra-articular bleeding leads to the development of chronic arthropathy. Other causes of joint pain include chromic synovitis, synovial hypertrophy, osteoarthritis and effusion. Point of care joint ultrasound is a newer technology that detects the etiology of joint pain, identifying the need for management factor concentrates or blood components. A repeat ultrasound detects the resolution of bleed and also helpful for beginning physiotherapy after excluding active bleeding. It is also effective in provided targeted management of underlying orthopedic abnormalities. It also provides precision to guide intra articular needle placement and is useful for long term follow up of joints Pakistan is a developing country where there is an acute shortage of factor concentrates. In this situation ensuring a joint bleed through point of care ultrasound will help in optimal use of resources.

Haemophilia is an ideal disease to target for gene therapy since it is caused by mutations in a single identified gene. A slight increase in factor activity can transform a severe hemophilic into a mild form. Current treatment with Plasma or recombinant FVIII or FIX is safe and effective but invasive, expensive and demanding. Gene therapy offers the potential for a cure by continuous endogenous expression of FVIII/FIX protein at therapeutic levels following a single administration of vector. Gene therapy has now been successful in adults with haemophilia A and B. Current trials are now ongoing in pediatric patients.

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SS-Haem-0011

MOLECULAR TESTING & BLOOD SAFETY

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ABSTRACT

Objective: To study the frequency of Nucleic Acid Test positivity in serologically negative hepatitis B virus, hepatitis C virus and human immunodeficiency virus in blood donors.

Study design: Cross sectional retrospective study.

Methodology: The study was conducted at Armed Forces Institute of Transfusion, Rawalpindi. Blood donors fulfilling the donation criteria were included in the study. Serological tests were performed on donated blood for HBsAg, Anti HCV and Anti HIV/p24. Positive samples were discarded and negative samples were tested by multiplex Nucleic Acid Testing (NAT) for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus.

Results: A total of 258,688 blood donors were screened for HBsAg, Anti HCV and Anti HIV/P24 and Anti-TP antibodies during study period. There were 12,325 (4.8%) positive samples on serology which were discarded. Remaining 246,363 samples negative on serology were subjected to multiplex NAT testing for HBV, HCV and HIV. Out of these, 109 (1 in 2260) donors were positive on NAT testing for HBV and HCV. Out of 109, there were 84 (1 in 2932) HBV and 25(1 in 9854) HCV positive cases.

Conclusion: NAT detected HBV and HCV which were missed by serology. Since every blood bag is separated into three components, NAT prevented 327 transfusion transmissible infections (TTIs) in 5 years. Introduction of NAT in blood banks is the way forward in countries with high burden of TTIs, such as Pakistan.

Key words: Nucleic Acid Test (NAT), Transfusion Transmitted Infections (TTIs), Blood donors

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SS-Haem-0012

APPLICATION OF MOLECULAR TESTING IN IMMUNE HAEMATOLOGY

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ABSTRACT

Understanding of genetic changes associated with most blood group antigen and phenotype enable us to identify blood group antigens and antibodies using necleic acid based approaches.

Haemagglutination tests for antigens still are the gold standard but has limitations e.g cannot indicate zygosity of 'D' antigens or relied upon to type recently transfused patients. With this background,the lecture will refer to molecular basis of blood group antigens, antigen identificaiton by DNA testing and clinical applications of DNA analysis for blood group antigens. These include

- To type patients who has been recently.
- To type patietnst whose RBCs are coated with immunoglobulin
- To identify a fetus at risk for hemoytic disease of the newborn
- To determine which phenotypically antigen-negative patients can receive antigen-positive RBCs.
- To type donors for antibody identificaiton panels
- To type patients who have an antigen that is expressed weakly on RBCs
- To determine RHD zygosity
- To mass screen for antigen-negative donors
- To resolve blood group A, B, and D discrepancies
- To determine the origin of engrafted leukocytes in a stem cell recipient
- To determine the origin of lymphocytes in a patient with graft-versus-host disease
- For tissue typing
- For paternity and immigration testing
- For forensic testing

Finally discuss briefly the present limitation of molecular testing.

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SS-Haem-0013

EXPERIENCE OF HEMOVIGILANCE AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Definition and types of hemo-vigilance practical in healthcare. Importance of HV in healthcare. Importance.

SS-Haem-0014

NEONATAL ALLOIMMUNE THROMBOCYTOPENIA

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ABSTRACT

Thrombocytopenia is a common hematological abnormality in Neonates. With its causes becoming clearer than ever now, treatments can be narrowed down depending on causes and the mechanisms behind it. It is also due to better understanding of mechanisms underlying Neonatal Thrombocytopenia that more logical and innovative treatments are being developed. The major mechanism is impaired function, with increased consumption and/or sequestration as a noteworthy second cause e.g. Neonatal Allo-immune Thrombocytopenia (NAIT), becoming increasingly important. Combined mechanisms of thrombocytopenia are also emerging as causes of significance in Neonatal thrombocytopenia.

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SS-Haem-0015

APLASTIC ANEMIA - EXPLORING THE GENETIC LANDSCAPE

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ABSTRACT

Acquired aplastic is a nonmalignant disease caused by destruction of early hematopoietic precursors. Most patients with severe aplastic anemia (SAA) respond to immunosuppressive therapy (IST), implicating an immune pathophysiology. However, malignant or "clonal evolution" to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) occurs in approximately 15% of cases. While possible mechanisms for this phenomenon include immune escape and increased proliferation the pathophysiology of this clonal evolution is not well understood.

The application of next-generation sequencing to de novo MDS/AML has led to definition of recurrently mutated genes as possible drivers of leukemogenesis. Do similarly mutated genes exist in those cases of aplastic anemia that undergo clonal evolution? If so, how early in the course of the disease can they be detected by modern molecular techniques? And can these changes inform and guide treatment strategies for the individual patient?

The aim of this paper is to look at the currently available evidence, analyze the genetic landscape of acquired aplastic anemia and attempt to explore its pathophysiologic link to clonality.

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SS-Haem-0016

DIAGNOSIS OF BETA THALASSAEMIA INTERMEDIA

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ABSTRACT

Thalassaemia Intermedia (TI) is a heterogenous group of genetic haemoglobin disorders that have a clinical picture ranging from little more than β-thalassaemia trait to slightly less than β-thalassaemia major. At one extreme there may be patients who have never received blood transfusions while at the other extreme there may be patients who may be getting regular blood transfusions. The extreme heterogeneity of TI is because of several genetic modifiers that may exist alone or in combination.

Traditionally TI is diagnosed on clinical grounds. Any patient who can maintain haemoglobin above 7.0 g/dL without blood transfusions or who does not require blood as frequently as a typical thalassamia major is labelled as TI. The level of Hb-F does not correlate with the severity of TI. Genetic analysis may be used to make a prospective diagnosis of TI. But considering the high cost of genetic testing it should seldom be required. In most situations a careful transfusion history should be enough to make the diagnosis.

The genetic basis of TI includes mild β-thalassaemia mutations (β^+). Most mild β^+ mutations are located in the promoter region of the β-globin gene, for example C-T substitution at -88 and -101 and A-C substitution at the Cap+1 site. Mutations in the poly-A tail of the β-globin gene, though rare, are also mild. These mutations when co-inherited with the β^o-thalassaemia mutations usually produce thalassaemia intermedia phenotype. Splice junction mutations (Cd 30, IVSI-1, IVSI-5, and IVSII-1 etc.) are severe β^+ thalassaemia mutations with some residual Hb-A synthesis. Homozygotes of the splice junction mutations also have a slightly less severe disease.

There are also many secondary genetic modifiers that can restore the imbalance between α-chains and β-chains. These include co-inheritance of different forms of α-thalassaemia, production of α-globin stabilizing factors, and enhanced production of Hb-F linked directly to the β-globin gene cluster (Xmn-I polymorphism) or located on some other chromosomes like BCL11A, KLF1, HBS1L-MYB etc.

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SS-Haem-0017

LESSONS LEARNT IN MANAGEMENT OF REGIONAL BLOOD CENTERS

Dr. Saba Jamal

SS-Haem-0018

MANAGEMENTS OF MPNS

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SS-Haem-0019

NEXT GENERATION SEQUENCING IN MYELODYSPLASTIC DISORDER

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SS-Haem-0020

HIGH GRADE B CELL LYMPHOMA; DOUBLE EXPRESSER VS TRIPLE HIT LYMPHOMA

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HAEMATOLOGY FREE PAPER ABSTRACTS

FP-Haem-0001

FREQUENCY OF CAP1+ MUTATION IN BETA THALASSEMIA & ITS ASSOCIATED HEMATOLOGICAL FEATURES

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ABSTRACT

Objective: To determine the frequency of Cap+1 mutation in beta thalassaemia and its association with haematological features

Study Design: Cross sectional study

Place and Duration of Study: Department of haematology, Armed Forces Institute of Pathology (AFIP) Rawalpindi from August 2017 to August 2018.

Material and Methods: Molecular genetics for Cap+1 mutation was done by extracting DNA from whole blood by using genomic DNA purification kit (Gentra system USA). Primers were designed for detection of normal and mutant DNA. Basic haematological parameters were performed using automated analyzer (Sysmex KX-21).

Results: The frequency of Cap+1 mutation was observed in 1.2% (5/421) in all suspected cases of beta thalassaemia with mildly low MCV and MCH.

Conclusion: Cap+1 mutation is a silent mutation and its diagnosis remains a challenge for haematologists because of its normal presentation and mildly deranged basic haematological parameters. Individuals with Cap+1 mutation may produce children with beta thalassaemia intermedia if they marry an individual with beta thalassaemia minor. Cap+1 mutation is an unsuspected cause of thalassaemia transmission in Pakistan but its detection at molecular level has revolutionized the thalassaemia prevention programme in Pakistan.

Key Words: Cap+1 mutation, Silent, Beta thalassaemia, Polymerase chain reaction.

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FP-Haem-0002

COMPARATIVE ANALYSIS OF CELLULOSE ACETATE HEMOGLOBIN ELECTROPHORESIS AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR QUANTITATIVE DETERMINATION OF HEMOGLOBIN A2

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ABSTRACT

Objective: The present study is designed to evaluate the reliability and cost effectiveness of cellulose acetate Hb electrophoresis and high-performance liquid chromatography (HPLC) in the determination of HbA2 levels.

Material and Methods: The test population comprised 160 individuals divided into four groups: normal individuals, β-thalassemia trait (BTT) patients, iron deficiency anemia (IDA) patients, and co-morbid patients (BTT with IDA). HbA2 levels determined using cellulose acetate Hb electrophoresis and HPLC were compared.

Results: HbA2 levels were found to be diagnostic for classical BTT using either method. In co-morbid cases, both techniques failed to diagnose all cases of BTT. The sensitivity, specificity, and Youden's index for detection of the co-morbid condition was 69% and 66% for HPLC and cellulose acetate Hb electrophoresis, respectively.

Conclusion: This study revealed that semi-automated cellulose acetate Hb electrophoresis is more suitable for use in β-thalassemia prevention programs in low-income countries like Pakistan. This technique is easily available, simple and cost effective.

Key Words: Cellulose acetate hemoglobin electrophoresis, Hemoglobin A2, High performance liquid chromatography.

FP-Haem-0003

HEMOGRAM INDICES OF HEALTHY LACTOVEGETARIAN POPULATION FROM A THARPARKAR VILLAGE

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ABSTRACT

Objective: The evidence of micro nutrients deficiencies related mortalities are emerging all over the globe. Lack of the knowledge of dietary source of vitamin B complex and iron affects the haemogram indices. Aim of this study was to assess the haemogram values of healthy lactovegetarian population of Tharparkar village and to correlate these with WHO parameters.

Study Design: Descriptive cross-sectional study

Material and Methods. This descriptive cross-sectional study was conducted in 2012-13 on 100 apparently healthy subjects of both genders with age 14 to 55 years. Peripheral smears were prepared using Leishman stain at the research field during sampling. Anti-coagulated whole blood samples were collected and transported to the Dow university lab at Karachi under proper temperature.

Result. The mean age of the subjects in this study was 30.5 (±8.3) and the male to female ratio was 2.1:1 The mean haemoglobin level was 13.5 (±1.6). Mean MCV level was 93.6 (±9.9) mean MCH was found as 33.9 (±3.1).and mean Haematocrit was found as 40.4. (±5.7)

Conclusion; The blood indices of lactovegetarian population of Tharparkar village fall within the specified range as set by WHO Parameters except MCV which was found higher than normal. This may be attributed to the deficiency of vitamin B12 or Folate.

Key Word: Lactovegetarian, Haemogram indices, vitamin B12/ Folate deficiency, Anemia

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FP-Haem-0004

THE EVOLVING LANDSCAPE OF MPNS

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ABSTRACT

Objective: To determine frequency of CALR, MPL and JAK2 exon 12 mutation in JAK2V617F negative patients and to study their association with clinico-haematologic features.

Study design: Cross sectional study

Material and Methods: The study was conducted at Haematology Department, Armed Forces Institute of Pathology from June 2017 to June 2018. All patients were diagnosed to have myeloproliferative neoplasm (MPN) according to WHO 2016 criteria. PCR for JAK2V617F was done on real time PCR.PCR for JAK2 exon 12, CALR and MPL W515K/L, was done by Sanger sequencing. Clinico hematological parameters were noted and compared.

Results: A total of 48 newly diagnosed MPN patients who were JAK2V617F negative were enrolled in the study. Median age was 43.5 years. 38 were males and 10 were females with M:F ratio of 3.8:1. Of these patients,22 were diagnosed as PV, 16 as ET, 7 as PMF and 3 as CML.

CALR mutation was detected in 4 (8.3%) of MPN cases whereas no MPL and JAK2 exon 12mutation was detected on molecular analysis.44 patients were triple negative.

Conclusion: We conclude that CALR mutation was present in JAK2V617 negative MPN patients. PMF shares a high rate of CALR mutation in comparison with CML, PV and ET.

Key Words: Calreticulin Gene, Myeloproliferative Neoplasms

FP-Haem-0005

VARIANTS IN CML: BREAKPOINT CLUSTER REGION ABELSON ONCOGENE THE EVOLVING LANDSCAPE OF MPNS

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ABSTRACT

Chronic Myeloid Leukemia is characterized by a balanced genetic translocation (9;22), involving a fusion of the Abelson oncogene (ABL) from chromosome 9 with the breakpoint cluster region (BCR) gene on chromosome 22. The molecular consequence of this translocation is the generation of a BCR-ABL fusion oncogene, which in turn translates into a Bcr-Abl oncoprotein. CML can be diagnosed with different tests including Blood counts, routine cytogenetics FISH and RT-PCR. RT PCR is by far the most reliable and efficient test for CML as the studies have shown high sensitivity and specificity of RT-PCR in qualitative and quantitative detection of BCR ABL. Minimal residual disease in patients under TKI therapy can also be determined efficiently by quantitative RT-PCR. The structure of the chimeric BCR-ABL mRNA differs according to the breakpoint in the corresponding genes. More than 95% of Ph-positive CML patients present a breakpoint in the M-BCR region at exon e13 (b2) or exon e14 (b3). The breakpoint in the m-BCR region results in an e1a2 junction. In this study, we aimed to quantitatively detect BCR ABL transcript in 48 CML patients and also to identify the frequency of different BCR-ABL transcripts in the patients using RT-PCR. Results of FISH, Bone Marrow test and Blood cell count were recorded from reports of patients and were correlated with RT-PCR detection of BCR ABL. RNA was extracted from plasma samples but RNA from blood cells samples of 25 patients was also extracted for the sake of comparison of mRNA extraction and detection in both types of samples. Results show that quality and quantity of RNA extracted from blood cells is much better than that of plasma. Mean RNA yield (ng/µl) from plasma samples was 15.82 ± 0.90 and that from PB cells samples was 35.47 ± 2.55. The BCR-ABL/G6PD ratio in PB cells and plasma showed a significant correlation ($p<0.05$) but BCR-ABL/G6PD ratios from plasma samples were low than that of PB cells samples. There was weak agreement between FISH and RT-PCR in detection of BCR-ABL translocation in CML patients (kappa= 0.16). Patients were checked for three types of BCR ABL transcripts using specific primers. The most common was b3a2, followed by b3a2 present in 32 (66.66%) and 10 (20.83%) respectively. Transcript e1a2 was not detected at all. No transcript was detected in 6 (12.5%) patients. No co-expression was found. No significant correlation was found between transcript type and hematological parameters ($p>0.05$). Also, no significant correlation of transcript type with gender and age was found ($p>0.05$).

FP-Haem-0006

ACUTE PROMYELOCYTIC LEUKEMIA: AN EXPERIENCE AT A TERTIARY CARE HOSPITAL IN PAKISTAN

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ABSTRACT

Objective: Acute promyelocytic leukemia (APL) is a unique subtype of AML. There are very limited data about APL from Pakistan. The aim of the present study is to evaluate the clinico-demographic profile along with risk stratification of APL at a tertiary care hospital in Pakistan.

Materials and Methods: Between June 2014 and July 2018, 28 patients with APL were enrolled in this descriptive cross-sectional study. All data were documented and statistical analysis was performed by SPSS-20 software.

Results: Median age was 21 (range 2-65) years. Male to female ratio was 3:1. Hyper granular variant (92.8%) was more common as compared to microgranular type (7.14%). Majority of patients had complaints of fever (71.4%), bleeding (53.5%) and generalized weakness (14.2%). Pallor (64.2%) was the predominant finding on physical examination followed by petechial and purpural rashes (46.4%). Mean Hemoglobin was 8.3 (range 5.3-12.2) g/dl. The mean total leukocytes count was 39.6 (range 1.3-121)

x 10^9/ L and mean platelet count was 40 (range 7-78) x 10^9 /L. Most patients fall into high risk group (60.7%) on risk stratification followed by intermediate risk (32.1%) and low risk (7.1%).

Conclusion: In the present study pallor is the most common presentation. Risk stratification shows predominance of high-risk score.

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FP-Haem-0007

PULMONARY COMPLICATIONS OF BLOOD TRANSFUSIONS REPORTED AT A LARGE TERTIAY CARE HOSPITAL

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ABSTRACT

Objective: We aimed to analyze the pulmonary complications occurring due to blood transfusions at a large tertiary care hospital.

Material and methods: A retrospective cross-sectional study was conducted at blood bank of Aga Khan University Hospital from January 2016 till September 2017. Pulmonary complications including TACO, TRALI and TAD were analyzed in detail by reviewing medical record chart of patients. Clinical and radiological findings were reviewed. Variables analyzed included age, gender, principal diagnosis, co-morbidities, type of blood component transfused, presenting symptoms and outcome.

Results: A total of 111,721 blood components were transfused with 163 reported transfusion reactions [Mild reactions 94.4% (n=154) and severe reactions 5.52% (n=9)]. Rate of transfusion reaction was 1.45 per 1000 blood products. Number of pulmonary complications reported was 11 (6.74%) with 8 cases of TACO, 2 possible TRALI and 1 TAD. Mean age was 42 years. Male to female ratio was 1.7:1. Breathlessness was the commonest presenting symptom. In all TACO cases, significant comorbidities like renal disease, heart disease, hypoalbuminemia, positive fluid balance and underlying pulmonary edema were identified. No mortality was associated with TACO; however, prolonged hospital stay occurred in 5 patients, and 2 patients required BIPAP support. In TAD, no comorbidity was present and patient recovered after supportive treatment. The mortality associated with TRALI was 50% (n=1)

Conclusion: TACO was the commonest pulmonary complication associated with transfusion. All patients with TACO had underlying precipitating co-morbidities .A formal pre-transfusion risk assessment should be performed for TACO as this complication can be prevented. TRALI was associated with significant mortality.

Key words: TACO, TRALI, TAD, BIPAP

FP-Haem-0008

PREVALENCE OF TTI AMONG HEALTHY BLOOD DONORS IN SOUTH PUNJAB

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ABSTRACT

Objective: The Objective of this study was to ascertain the prevalence of Transfusion Transmissible Infections among the blood donors in South Punjab

Study Design: A Prospective Cohort Study

Place and Duration: This Study was conducted from Oct 2017 to Sept 2018, at the Regional Blood Centers of Bahawalpur and Multan, being managed by The Indus Hospital Network. Donors were carefully interviewed by trained health professionals and were screened for Hepatitis B, Hepatitis C, Human Immunodeficiency Viruses and Syphilis by Chemiluminescent Micro particle-based Assay method on Architect Plus i2000 SR (Abbot Diagnostics, USA) while Malaria by immunochromato-graphic (ICT) method

Results: Out of a total of 122,796 potential donors who were interviewed, 22,432 (18.27%) were deferred for various reasons and 100,364 donors were drawn. A total of 10,701 (10.66%) donations were found to be reactive. The prevalence of Hepatitis B, Hepatitis C, HIV, Syphilis and Malaria was found to be 2984 (2.88%), 5638 (5.61%), 126 (0.12%), 2021(2.01) and 22 (0.02%) respectively

Conclusion: A frightening percentage of people who considered themselves healthy enough to donate blood were reactive to TTIs in South Punjab with potentially many more still going undiagnosed. Among the various causes, unsafe blood transfusion is a major cause and there is an urgent requirement of streamlining and strengthening them on the lines of International standards including addition of NAT testing

HAEMATOLOGY POSTERS PRESENTATION ABSTRACTS

P-Haem-0001

Spectrum of Snake bites: Hematological & CNS complications and treatment outcomes in cases of Ophitoxaemia: An experience at a tertiary care hospital

Shaheena Butt

ABSTRACT

Objective: Objective of this study is to highlight the spectrum of haematological, central nervous system complications and treatment outcome in cases of ophitoxaemia.

Materials and Methods

Type of Study: Descriptive Analytical Study

Place of Study: Combined Military Hospital Kharian

Study Period.: All cases of ophitoxaemia admitted in Combined Military Hospital (CMH) Kharian cantonment from June 2007 to November 2008

Results During the study period a total of fifty cases of snake bites were admitted in CMH Kharian. These cases reported between June 2007 to Nov 2007 and then between June 2008 to Nov 2008. No case of snake bite reported from December 2007 till May 2008. There were 33 (66%) male and 17 (34%) females. The mean age was 34.8 years. The mean hospital stay was 5.06 days (maximum 13 days). There were 34 events of haematological complications and nine patients developed central nervous system (CNS) complications including respiratory failure in seven patients warranting mechanical ventilator support. Antivenom (AV) was administered to 32 patients only. Dose of AV ranged from 40-200 ml with a mean value of 37.29 ml. The administration of AV was associated with allergic febrile reactions in twenty-five patients (78.1%). In this study four patients died with mortality rate of 8%. The bite to needle time in the patients with fatal outcome was > 24 hours.

Conclusion: Snake bites are still associated with fatal outcome, mainly because of late administration of AV. Successful management of hematological and central nervous complications requires the availability of facilities for mechanical ventilator support and blood components.

P-Haem-0002

COMPARISON OF EFFICAY AND SAFETY OF IMATINIB VS NILOTINIB AS FIRST-LINE THERAPY IN NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA

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ABSTRACT

Objective: This study was conducted to investigate efficacy and safety of nilotinib 300 mg twice daily vs imatinib 400 mg once daily in Pakistani population.

Results: The primary end-point was met with a statistically significant higher rate of cytogenetic response (0% Philadelphia chromosome–positive [Ph+] metaphases by standard cytogenetics) at 12 months in the nilotinib arm vs the imatinib arm (94% vs 79%; $P < .01$). The safety profiles of both drugs were similar to those from previous studies.

Conclusion: In conclusion, rates of CCy(complete cytogenetic response) at 12 months were superior with nilotinib vs imatinib in Pakistani patients with newly diagnosed Ph+ CML-CP. To the best of our knowledge there are no published randomized or observational head to head trials of imatinib and nilotinib in newly diagnosed chronic phase CML in Pakistan.

Key Words: Chronic myeloid leukemia, first-line tyrosine kinase inhibitor, efficacy, safety

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P-Haem-0003

THE PATTERNS OF BLOOD GROUP A1 AND A2 IN PAKISTANI POPULATION: A TERTIARY CARE CENTER EXPERIENCE

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ABSTRACT

Objective: The objective of our study was to determine the prevalence of A_1 and A_2 subgroups among the donor population of Pakistan.

Study: Descriptive Cross-Sectional Study

Material and Methods: A prospective cross-sectional study was conducted at Armed Forces Institute of Transfusion, Rawalpindi from September 2017 till December 2017, after approval from ethical committee. The blood samples of blood donors were collected in EDTA bottles after informed consent. Blood grouping for all blood donors was performed on semi-automated analyzer Qasar IV. Blood groups were interpreted based on the agglutination pattern of forward and reverse grouping. The donors with blood group A and AB were included in the study and were typed for A_1 and A_2 subgroups with the help of anti-A_1 lectin. The Donors with blood group B and O were excluded from the study. The serum of all individuals with blood groups A_2 and A_2B was also tested for the presence of anti-A_1 with the help of A_1 cells.

Results: A total of 4485 donors reported during study period. Out of these, 1538/4485 (34.29%) had blood group A and AB. Further analysis of the data of study population revealed that 1116/1538 (72.56%) had blood group A and 422/1538 (27.43%) had AB. Out of 1116 donors with blood group A, 146/1116 (13.08%) had subgroup A_2 and 80/422(18.95%) donors with blood group AB were A_2B. Anti A2 antibody was present in 21/146 (14.38%) donors with A_2 blood group and 2/80(2.50%) of donors with A_2 B blood group.

Conclusion: Blood group A_2 is quite prevalent in our population. Although anti-A_1 is present in a significant number of individuals with blood group A_2, but there is no need to identify the A_2 blood group routinely. Multi-center

study with larger number patients is required to see the hidden aspects of this disease.

Key Words: ABO blood grouping, A_1 blood group, A_2 blood group

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P-Haem-0004

AUTOIMMUNE CYTOPENIAS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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ABSTRACT

Objective: To determine the frequency of autoimmune cytopenias in Chronic lymphocytic leukemia.

Study Design: Cross sectional study.

Place and Duration of Study: January 2018 to September 2018, Department of Haematology Army Medical College Rawalpindi in collaboration with Military Hospital Rawalpindi and Armed Forces institute of Pathology Rawalpindi.

Material and Methods: All patients of Chronic Lymphocytic Leukemia without previous known autoimmune disease, previous transfusion reactions or non- recipients of chemotherapy during previous one month were included in the study. Age and gender of the patient was noted. Complete blood counts, peripheral film examination, reticulocyte count, direct coombs test, serum LDH, direct and indirect bilirubin, bone marrow examination, anti-neutrophil cytoplasmic antibody test, RA factor, anti-nuclear antibody tests were done. Sampling technique was non-probability purposive sampling. Data was analyzed by SPSS 23 version. Frequency of autoimmune hemolytic anaemia, immune thrombocytopenic purpura, pure red cell aplasia and autoimmune agranulocytosis were noted.

Results: A total of 64 patients of chronic lymphocytic leukemia were included in the study, these were 53 males (82%) and 11 females (17.2%). Mean age of patients was 65 years. Autoimmune hemolytic anaemia was seen in 5/64 (7.8%) of patients. ITP was seen in 2/64 (3.1%) patients. Autoimmune granulocytopenia and pure red cell aplasia were not detected in our study population.

Conclusion: Autoimmune hemolytic anaemia and immune thrombocytopenic purpura are the most common causes of cytopenias in patients with Chronic lymphocytic leukemia. They should always be screened for by laboratory tests, as their management is different from other complications which occur due to bone marrow infiltration in chronic lymphocytic leukemia.

Key Words: Chronic lymphocytic leukemia, Autoimmune hemolytic anaemia, Immune thrombocytopenic purpura.

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P-Haem-0005

DIFFERENTIATE B12 DEFICIENCY ALONE FROM COMBINED B12-IRON AND B12- BETA THALASSAEMIA TRAIT ON THE BASIS OF HEMATOLOGICAL PARAMETERS AND PLT/MCH RATIO

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ABSTRACT

Objective: To compare the hemogram indices of solo vitamin B12 deficiency with combined B12-iron,B12-beta thalassaemia trait and B12-RBC Folate deficiency.

Study design: Cross sectional study design

Material and Method: Clinical records of one hundred and five vitamin B12 deficient cases (B12 less than 200ng/l) who had presented with normal or low MCV (MCV<95fl) on complete blood count were determined from Dow Diagnostic Research and Reference laboratory. Serum ferritin, red blood cell folate level and Hb electrophoresis for beta thalassaemia trait were analysed in these patients.

Result: Significant differences in the mean values of Hb, MCH and MCHC were found. They were decreased in combine deficiencies as compare to solo B12 deficiency. Considerable number of cases of combine deficiencies showed anemia with microcytic blood picture while platelet count and PLT/MCH ratio were increased in combined deficiency group.

Conclusion: It was concluded that combined deficiencies masked the morphological changes of B12 deficiency. We proposed that we can analyse the hemogram indices and PLT/MCH ratio for differentiating B12 deficient group alone from combine B12-iron,B12-beta thalassaemia trait. We suggest that physicians must keep in mind the combined deficiencies with B12 in their differential diagnosis for a relatively wide spectrum of cases.

Key words: vitamin B12, iron deficiency, β-thalassaemia trait

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P-Haem-0006

PREVALENCE OF HEPATITIS B AND HEPATITIS C IN MULTITRANSFUSED BETA THALASSEMIA PATIENTS

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ABSTRACT

Objective: Beta thalassemia major is common inherited autosomal recessive disorder in Pakistan and regular blood transfusions are necessary to prevent severe anemia and physical changes. Due to repeated blood transfusions hepatitis B and hepatitis C infections occur. The objective of this study was to evaluate the prevalence of hepatitis B and C in multi transfused beta thalassemia patients.

Material and Methods: This is a descriptive cross-sectional study performed in thalassemia centre Holy family hospital Rawalpindi in collaboration with department of pathology Holy family hospital Rawalpindi. 300 patients were selected and interviewed about age, age at time of diagnosis, frequency of transfusion, and present clinical status. A thorough physical examination was done. Blood samples were collected by standard phlebotomy technique. Samples were processed in pathology lab, Holy Family hospital Rawalpindi. Diagnosis of beta thalassemia was confirmed by standard Hb electrophoresis. Blood samples were obtained for detection of HBsAg and Anti HCV using ELISA. Positive results were confirmed using PCR.
Results: 300 patients of thalassemia aged < 30 years were enrolled in this study with 1:1 ratio of male to female. Study population had 10% positivity for HBV and 34% positivity for HCV. 20.3% (n=61) had splenomegaly and 19% (n =56) hepatomegaly. Consanguineous marriage was found in 83% of case with 19.3% of sibling history positive for major and intermedia.
Conclusion: Most of the patients were suffering from HCV and HBV at the same time. A huge age range of thalassemic patients were susceptible with HCV. Enlargement of liver and spleen were not common clinical characteristics in thalassemic patients with HCV and HBV.
Key Words: Hepatitis B, Hepatitis C, Thalassemia Major, Blood Transfusion.

P-Haem-0007

FANCONI ANEMIA PRESENTING LATE AS MALIGNANT ORAL CARCINOMA: A RARE CASE FROM PAKISTAN

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ABSTRACT
Objective: To present a case of Fanconi anemia presenting late as oral squamous cell carcinoma
Study Design: Case Report
Case Summary: Fanconi Anemia is a rare autosomal recessive disorder characterized by various congenital malformations, progressive bone marrow failure usually in young age and solid tumor development. We present a case where the patient presented at a very late age with a solid tumor. A 50-year-old unmarried lady was referred to hematology OPD of Armed Forces Bone Marrow Transplant Centre Rawalpindi with low blood counts in all three lineages, she developed mass left buccal mucosa. Excisional biopsy gave a diagnosis of Squamous Cell Oral Carcinoma. The appearances on CECT were consistent with Squamous Cell Carcinoma of Buccal Mucosa. Enlarged metastatic lymph node was detected at level I-b. Tumor was staged as T4b, N1, Mx. Further assessment at hematology clinic revealed that she is an unmarried lady, history of easy bruising and multiple dark spots at the trunk. On general physical examination she had pallor, cervical lymphadenopathy at the level of left I-b, microcephaly, micrognathia, left side oral ulcer involving Stenson's duct, and left sided non-tender facial swelling, stiff low set thumbs, hypo plastic radii with weak pulses, multiple café au late & hypo pigmented spots on body. Bone marrow Aspirate revealed reduced erythropoiesis, myelopoiesis, megakaryocytes with prominent lymphocytes and plasma cells and increased iron stores. Bone marrow trephine biopsy was consistent with earlier findings of bone marrow aplasia. There was no evidence of atypical infiltrate in the bony fragment evaluated. A diagnosis of Fanconi anemia presenting as Malignant Oral CA was made after clinic-pathological correlation.
Conclusion: We present first case of Fanconi anemia with late presentation at the age of 50 years to the best of our knowledge.
Keywords: Fanconi anemia, Oral carcinoma, Squamous Cell Carcinoma, late presentation.

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P-Haem-0008

AMYLOIDOSIS INVOLVING BONE MARROW WITHOUT MONOCLONAL GAMMOPATHY: A RARE ENTITY

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ABSTRACT
Amyloidosis is extracellular deposition of fibrillar proteins in various tissues of body. Bone marrow involvement is common and presents as multiple myeloma. We present case of 54-year-old male who present with in infiltration of amyloidosis in bone marrow but without any monoclonal gammopathy having no evidence of plasma cells on bone marrow aspirate or trephine biopsy, normal protein and urine electrophoresis.

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P-Haem-0009

PRIMARY HEMATOLOGICAL DISORDERS, LEADING CAUSE OF RARE BUDD-CHIARI SYNDROME; DATA FROM LARGE TERTIARY CARE HOSPITAL

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ABSTRACT
Objective: To determine the frequency and pattern of hematological abnormalities leading to BCS at a tertiary care hospital.
Material and Methods: It was a retrospective cross-sectional study conducted at Aga Khan University Hospital during January 2013 till December 2017. Diagnosed cases of budd-chiari syndrome were included. Data obtained include mean age, gender, presenting symptoms, risk

factors, laboratory parameters, other thrombosis, type of treatment and outcome.

Results: A total of 50 patients presented with BCS during study period. Mean age was 32 years [(8% pediatric (n=4), 92%adult (n=46)]. Male to female ratio is 1.7:1. Commonest presenting symptom was abdominal distention (76%) followed by jaundice (24%). In 26 % (n=13) underlying risk factor was unknown. Among 74%(n=37) risk factors include hereditary thrombophilia 22%(n=11), hepatocellular carcinoma 14%(n=7), antiphospholipid syndrome (APLA) 8%(n=4), polycythemia rubra vera (PRV) 8%(n=4). Mean Hb: 11g/dl, WBC: 11 x 10^9/L, PLT: 206 x 10^9/L, PT/INR: 17.2/1.8, APTT: 32.9 seconds at presentation. Mean protein C: 44.4%, protein S: 62%, antithrombin III: 57.9% and factor V leiden: 0.5 in 48% (n=24) patients. 4 patients with hereditary thrombophilia had previous thrombosis including deep venous thrombosis (DVT) (n=3) and cerebral sinus thrombosis (n=1). 5 patients had thrombosis at other sites along with BCS which includes DVT, pulmonary artery thrombosis, common iliac vein and portal venous thrombosis. Medical treatment includes enoxaparin in 58 % (n=29) followed by warfarin in 20 % (n=10) and 22% (n=11) no treatment was provided. Surgical treatment was given in only 22% (n=11). 36% (n=18) were died, 20 % (n=11) are alive and 44% (n=22) were lost to follow up.

Conclusion: Primary hematological disorder including hereditary thrombophilia, APLA and PRV are leading risk factors in BCS. Mortality was observed in 22% of patients.

Key words: BCS, PRV, APLA, DVT

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P-Haem-0010

NATURAL KILLER CELL LEUKEMIA: A RARE CASE

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ABSTRACT

Aggressive natural killer cell leukemia/lymphoma is a rare and highly aggressive NK cell neoplasm with a short clinical course and poor prognosis, which is almost always EBV related. It often affects young patients and is characterized by relative resistance to standard chemotherapy. Here we report a case of 62 years old male who presented to us with complaints of fever, constipation, and generalized body aches for the past one week. His diagnosis of NK cell leukemia was made on having characteristic cells on peripheral film and with the help of flow cytometry showing strong positivity for CD56 confirming natural killer cell leukemia. It was rare one, as his age falls into the chronic type but he presented with an acute one with a short history and aggressive nature of disease.

Results: Immunohistochemistry was performed on bone marrow trephine and with the help of flow cytometry this case was diagnosed as natural killer cell leukemia.

Conclusion: A final diagnosis for leukemia's should always be made by combining the results of the morphology, immune- phenotyping, and immunohistochemical analysis correlating with clinical presentation.

Key Words: Bone marrow, Immunohistochemistry, Natural killer cells, CD56

P-Haem-0011

CLINICOHEMATOLOGICAL PATTERN OF HAEMATOLOGICAL MALIGNANCIES IN PATIENTS REFERRED FOR BONE MARROW EXAMINATION AT HOLY FAMILY HOSPITAL

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ABSTRACT

Objective: Aim of this study was to determine frequencies and clinicohematological features of different hematological malignancies in the patients presented in the Hematology department of Holy Family Hospital.

Materials and methods: Out of 1080 cases of bone marrow biopsies done at HFH, 284 patients with hematological malignancy were selected from September 2011 to September 2017. A systematic review of causes, clinical and bone marrow findings was done. Detailed history, clinical examination and hematological parameters at presentation were recorded. Bone marrow aspiration and trephine biopsy were carried out as per the clinical indication.

Results: 15 to 80 years old patients were enrolled with a male to female ratio of 2:1. In our study, the prevalence of hematological malignancies came out to be 24%. It was revealed that CML was the most common among all leukemias found in our setup.

Conclusion: Acute leukemias were common in younger age whereas chronic leukemias and lymphoproliferative disorders in more advanced ages.

Key Words: Hematological malignancy, Acute leukemia, Chronic leukemia, Lymphoproliferative disorders

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P-Haem-0012

HEMATOLOGICAL CHANGES IN DENGUE FEVER

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ABSTRACT

Objective: To assess the hematological parameters of patients with dengue fever so as to increase the sensitivity of screening and to use those parameters as an indicator to predict the severity of the disease.

Study design: Descriptive Retrospective Study.

Place and duration: This study was conducted in, HFH, Rawalpindi from September 2016 to December 2016. A total of 318 seropositive dengue patients were included.

Materials and Method: Patients were classified into two groups: severe dengue (SD) and dengue hemorrhagic fever (DHF). Following parameters were considered as hematological profile: RBC Count, WBC Count, Hemoglobin, Hematocrit, MCV, MCH, MCHC, Platelet Count, RDW, PDW, Lymphocyte % and Neutrophil %. Data was analyzed using SPSS v 22.0. Kolmogrov Smirnov and Shapiro Wilk tests were applied to confirm the normality of data distribution. Independent Sample "t" test at 5% confidence level was applied to develop a relation between hematological parameters and severity of disease.

Results: The Data was normally distributed. Mean age for SD and DHF was 33.46 and 31.61 respectively. The difference was statistically significant. There was a significant relation between WBC count, Platelet Count, MCV and the severity of the disease with the P value 0.04, 0.05 and 0.00 respectively.

Conclusion: Highly statistical association was found suggesting that these parameters can be used as an indicator to predict the severity of the disease.

Key Words: Dengue, Profile, Changes

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P-Haem-0013

PREVELANCE OF PANCYTOPENIA/ BICYTOPENIA IN PEDIATRIC POPULATION AND ITS ASSOCIATION WITH ETIOLOGY

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ABSTRACT

Objectives: Pancytopenia/Bicytopenia is an important clinico - haematological entity resulting from a number of disease processes that primarily or secondarily affect the bone marrow. The objectives of this study were to determine the prevalence of cytopenias and its association with age, gender distribution, clinical presentation and etiology in pediatric population presenting to a tertiary care hospital in Rawalpindi.

Study design: It was a descriptive cross-sectional study

Material and Method: This study was conducted in the department of haematology, Holy Family Hospital, Rawalpindi. 253 cases referred for bone marrow examination from 1st June 2013 to 31st may 2017 were included in the study using non-probability consecutive sampling. Out of these 108 patients were found to have bicytopenia and 108 had pancytopenia. Information regarding mode of presentation, clinical examination, complete blood counts and bone marrow examination was obtained. SPSS 21 was used to analyse the data, descriptive statistics were calculated. At univariable analysis, the clinical and etiological profile compared with cytopenias (Bicytopenia/pancytopenia) by using chi-square test. P value 0.05 was considered significant.

Results: Biytopenia was found in (133)52.6% patients and pancytopenia was found in (120)47.4% patients out of 253 referrals for bone marrow examination. Age of the patients ranged from 0 months-12years with a mean age of 5.5years. Out of 253 cases, 139(55%) were males, 114(45%) were females. The commonest presenting complaints were fever 225(89%), bleeding manifestations 52(20.5%) and bruises 36(14.2%). Pallor 234(92.4%) was the commonest finding on examination followed by hepatomegaly 118(46.64%) and splenomegaly 114(45.05%). The predominant finding on complete blood count was bicytopenia 133(52.6%) followed by pancytopenia 120(47.4%). Acute leukemia (28.5%) was the commonest cause of bicytopenia/pancytopenia followed by Megaloblastic anemia (17.4%) and Aplastic anemia(11.5%)

Conclusion: Bicytopenia/Pancytopenia was found to be a common cause of referral for bone marrow examination. Acute leukemia, Megaloblastic anemia and Aplastic anemia were found to be the common underlying etiologies.

Key Words: Pancytopenia, Bone marrow, Megaloblastic anemia, Aplastic anemia, Acute leukemia, Hypersplenism

P-Haem-0014

DISTRIBUTION PATTERN OF ABO GROUP AND RH GROUP AMONG DONORS IN SOUTH PUNJAB REGION; A STUDY OF TWO REGIONAL BLOOD CENTERS

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ABSTRACT

Objective: The objective of study to determine distribution pattern of ABO and Rh blood among donors in south Punjab region.

Study design: Retrospective study

Material and Methods: The study was carried on 100921 voluntary and replacement donors from October 2017 to September 2018 in regional blood center Multan and region blood center Bahawalpur. Samples from donor were drawn after aspetic standard technique and Blood group of the blood donors was determined by forward and reverse methods with commercially available standard monoclonal antisera by test tube agglutination techniques. Results were reported in number and percentages.

Results: From 100921 donors, blood group B is commonest in 35277 donors (34.94%) followed by group O in 31783 donors (31.49%), group A with 24539(24.31%) and group AB is minimum in 9322 donors (9.23%)

Whereas 93098 donors (92.25%) were Rh positive and 7823 donors (7.75%) Rh negative.

Conclusion: According to this study, blood group B positive is commonest one and AB negative least one. This study has given information about ABO and Rh blood group distribution in south Punjab and will help better management of regional blood transfusion services, motivation of voluntary donors and strategies for blood collection.

Key Words: ABO blood group, rhesus blood group, blood donor

P-Haem-0015

PATTERN OF HEMATOLOGICAL DISORDERS ON BONE MARROW EXAMINATION: A TERTIARY CARE HOSPITAL EXPERIENCE

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ABSTRACT

Objective: The aim of the study is to evaluate the clinical profile, spectrum, cytological and histological pattern of various hematological disorders reported in bone marrow aspiration and trephine biopsy respectively.

Study Design: This was a Retrospective and prospective study. This study was carried out in the Haematology Department of CHK Laboratory from July 2016 to August 2018.

Material and Methods: Bone marrow examination of 147 cases of suspected hematological disorders was carried out, who presented to CHK laboratory for bone marrow Biopsy. Complete details of history, examination and complete blood counts were
Recorded.

Results: Among 147 cases studied, age of patients ranged from 02 to 75 yrs with mean age of 38.5 yrs and male predominance (1.3:1). Most of the patients presented with fever, Easy fatigability and generalized weakness. Out of 147 cases of bone marrow biopsy 21.7% cases showed Normal haematopoiesis. Erythroid Hyperplasia in 5.4% cases and Megaloblastic Anaemia in 2% cases. ITP in 3.4%, Hypocellular marrow in 4%, Aplastic Anaemia in 9.5% cases. Acute leukemia was seen in 28 cases with 12.9% cases of ALL and 6.1% cases of AML. CML was seen in 14.2%, MPN in 4% cases, LPD in 9.5% cases, Multiple myeloma in 1.3%. 01 case (0.6%) of granulomatous pathology and 01 case (0.6%) of Myelodysplastic syndrome were diagnosed exclusively on bone marrow biopsy. In addition, metastatic deposits of adenocarcinoma were observed in 1.3% cases.

Conclusion: The present study showed the usefulness of bone marrow aspiration and trephine biopsy in evaluation of the bone marrow in routine haematological disorders and also for understanding disease progression.

Key Words: Leukemia, Haematological disorders, Bone marrow biopsy

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P-Haem-0016

RENAL FUNCTIONS AND THALASSEMIA TRAIT

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ABSTRACT

Objective: Persons with beta-thalassemia minor usually are symptomless. The aim of this study is to investigate renal function in patients with beta-thalassemia minor.

Study Design: Retrospective case control study.

Material and Methods: 200 subjects with beta-thalassemia minor and 200 sex- and age-matched without any trait were enrolled in the study. Blood and 24-hour urine samples were checked for hematologic and biochemical analysis.

Results: 32 (16%) patients had renal calculus. 48 (24%) patients had hypercalciuria, 36 (18%) patients had high creatinine clearance.

Conclusion: In summary, renal hyperfiltration, hypercalciuria, and albuminuria are common in thalassaemia trait subjects.

Key Words: Thalassemia trait, Renal functions, hypercalciuria, creatinine clearance

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IMMUNOLOGY SCIENTIFIC SESSION ABSTRACTS

SS-Imm-0001

SERUM FREE LIGHT CHAINS AND THEIR CLINICAL SIGNIFICANCE

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ABSTRACT

Serum Free Light Chains (sFLC), are highly specific tumour markers for a disease which usually remains hidden in the bone marrow. The laboratory test for FLC needs to be highly sensitive are highly specific with a consistent qualitative result to be able to provide clinically useful diagnostic information, in addition to the provision of an effective disease monitoring tool for lymphoproliferative disorders associated with the production of monoclonal immunoglobulins or light chains. Free light chains are filtered through glomeruli and reabsorbed almost completely in the proximal tubules in kidneys so testing for FLCs in urine is not useful until the reabsorption mechanism in kidneys is overwhelmed. The availability of a test for sFLCs with the above-mentioned characteristics has provided an effective tool for early and specific diagnosis of Light Chain Multiple Myeloma (LCMM), Smoldering Multiple Myeloma (SMM), Oligo-secretory Multiple Myeloma (OSMM), Intact Immunoglobulin Multiple Myeloma (IMM), Monoclonal Gammopathy of Unknown Significance (MGUS) and Light Chain Associated Amyloidosis. In addition, the ability to assess haematological responses after treatment has contributed to a steady improvement in prognosis for patients with plasma cell dycrasias. The clinical utility of sFLC tests is best studied in reference to their reference range in healthy population and a modified reference range as expected in patients with renal disease and in polyclonal gammopathies. The talk will emphasize local experience as regards the interpretation as a sensitive test is also highly vulnerable to prozone and instrumentation related false positive and negative results.

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SS-Imm-0002

THERAPEUTIC ROLE OF ANTI ADHESION MOLECULE ANTIBODIES

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ABSTRACT

The diversity of adhesion molecules especially integrins that play a complex role in many diseases suggests great potential for these molecules as drug targets. Exploration of the selective role for distinct leukocytic integrins indicated that homing of inflammatory cells to select disease sites depends on a highly regulated expression of discrete integrins and their ligands in limited locations. Two particular adhesive interactions: α4-integrin/MAdCAM-1 and β2-integrin/ICAM-1 are being targeted for Inflammatory Bowel Disease (IBD). A humanized monoclonal antibody against human α4 integrin is approved for the treatment of patients with active Crohn Disease (CD). This agent represents an efficacious therapeutic option for patients who do not respond to or have failed a TNF-α inhibitor. Vedolizumab produces few systemic adverse effects because it acts on gut-trophic α4β7 integrin and has been approved and is being used to treat IBD. Currently several anti-integrin drugs including etrolizumab which acts on β7-integrin which targets mucosal address in cell adhesion molecule-1, are undergoing clinical trials

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SS-Imm-0003

DESENSITIZATION PROTOCOL IN HIGHLY SENSITIZED TRANSPLANT RECIPIENTS

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ABSTRACT

Objective: Sensitization to HLA antigens in recipients is a major barrier to their successful transplantation. Protocols for desensitization have been developed using techniques of HLA antibody removal, antibody competition, depletion of antibody producing cells and sensitive techniques to detect antibodies. The objective of our study was to develop a workable protocol in the setting of an emerging economy performing living related donor transplants.

Study Design: This is a prospective study where highly sensitized recipients were desensitized using a combined removal, dilution and B cell depleting immunosuppression protocol.

Material and Methods: Seven highly sensitized patients with positive CDC, T and B cell flow cross-match positive and with Donor Specific Antibodies (DSA) detected by Luminex were desensitized according to SIUT protocol. This consisted of 7-10 sessions of plasmapheresis depending on antibody titre, low dose IVIg 100mg/Kg, Rituximab (Anti CD20) 375/m2 and Bortezomib 1-3mg/m2.

Results: Seven patients with DSA have been successfully desensitized and transplanted. Acute cellular rejections were encountered in 3 (42%) and antibody mediated

rejection in 1 (14.2%). In the follow-up period of 2 years all grafts are functioning and two patients have low titre DSA.

Conclusion: Advances in HLA antibody detection techniques, development B cell depleting antibodies and antibody removal has opened a new window of opportunity for transplant of high sensitized. Our results are encouraging in the short-term follow-up.

Key words: Desensitization, highly sensitizing protocol.

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SS-Imm-0004

STEM CELL APPLICATIONS: NEW HORIZONS IN CLINICAL IMMUNOLOGY

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ABSTRACT

The remarkable potential of stem cells to differentiate into specific cell types and their immunomodulatory properties continue to hold great promise for the treatment of many debilitating diseases. Recent years have seen remarkable progress in development of stem cell-based treatments and their applications as clinical standard of stem cell-based therapies provide new treatment approaches for the treatment of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and type 1 diabetes. This talk will focus on some of the basic research studies and experimental stem cell therapies for autoimmune diseases being guided by adult and embryonic stem cell discoveries

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SS-Imm-0005

IMMUNE ASPECTS OF ZIKA VIRUS INFECTION AND DISEASE

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ABSTRACT

Zika virus is categorized under the virus family *Flaviviridae* and the genus Flavivirus. The virus is transmitted via *Aedes* mosquitoes, such as *A. aegypti.* Human Zika-virus infection is begun when a blood-feeding female Aedes mosquito deposits the virus into human skin and the blood stream. Zika virus generally infects the skin dendritic cells and dermal fibroblasts. The expression of pathogen recognition receptor (PRR), toll-like receptor (TLR) RIG-1 and MDA-5 which subsequently trigger the expression of type 1-IFNs, IFN stimulated genes including OAS2, ISG-15 and MX-1 and inflammatory cytokines are up regulated by infection of dermal fibroblasts with Zika virus. Types 1- and 2-IFNs are known to be important for control of other flavi viruses' infections. Both types of IFNs inhibit replication of Zika virus in human fibroblasts. Studies have indicated that ZIKV evades the human type I IFN response suggesting a role for the adaptive immune response in resolving infection.

Serological analysis of patients with Zika virus disease demonstrates both anti-Zika-virus IgG and IgM and neutralizing antibodies, which may provide partial protection against lethal Zika virus infection. However, the extensive cross-reactivity of patient's serum antibodies against closely related flavi viruses, such as DENV and YFV frequently make unreliable, serologic laboratory results to diagnose Zika virus infection. Polyfunctional T-cell responses are induced upon Zika virus infection, however the role of the peripheral T cells remains unidentified. Thus, neutralizing antibodies provide partial protection whereas type 1- and type 2-IFNs are important in controlling Zika virus infection.

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SS-Imm-0006

TRANSPLANT INFECTIONS AND REJECTIONS: A DIAGNOSTIC AND THERAPEUTIC PERPLEXITY

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ABSTRACT

With the advent of new and more potent immuno-suppression, graft survival after renal transplant has improved due to a reduction in post-transplant rejections. However, this has led to emergence of severe infections. Both these complications of infections and rejections during synergize each other. Most of the infections when stimulate immunity in patients on immunosuppression, they lead to further imbalance in the system and loss of tolerance. This may lead to rejection. Also, augmentation of immuno-suppression to treat rejection increases susceptibility to infections.

Due to overlapping clinical and laboratory features for infections and rejections, diagnosis is delayed which is a dilemma in these patients. Effect of infections due to certain microorganisms such as cytomegalovirus is already known in causing rejections in solid organ transplant recipients. In this study effects of some viruses on renal graft have been highlighted that can cause immune dysregulation. Studying these mechanisms of graft dysfunction due to between over and under immunosuppression. This may both graft and patient survival

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SS-Imm-0007

ADVANCES IN TUMOR IMMUNOLOGY

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ABSTRACT

Treatment of cancers, by tradition, rests on three pillars: chemotherapy, surgery and radiation therapy. In the past twenty years, targeted therapies using drugs that target cancer cells bearing specific molecules have been recognised as standard treatment for many cancers, with Gleevec (imatinab) and Herceptin (trastuzumab) epitomising the success. After some setbacks, cancer immunology is now living its Golden Age. Recent advances in cancer immunology have provided new therapeutic approaches to treat cancer. Antibodies that block the immune checkpoints eg cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell-death protein 1 (PD-1)/programmed cell-death 1 ligand 1 (PD-L1) pathways, have been approved by FDA for the treatment of melanoma and anti-PD-1 antibody, nivolumab, received the FDA-approval in March 2015 for squamous lung cancer treatment. In addition, such treatments have shown exciting promise in the treatment of non-small cell lung carcinoma, renal cell carcinoma, bladder cancer, and Hodgkin lymphoma. Simultaneously, the field of immunotherapy has exploded to establish itself as the fifth pillar of cancer treatment. The chimeric antigen receptor (CAR) T cell technology has provided strong evidence of efficacy in the treatment of B cell malignancies, and different T cell based treatments are currently under investigation for different types of tumors. The presentation discusses the latest advances in cancer immunology and immunotherapy strategies that have shown encouraging results in preclinical models and clinical practice.

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SS-Imm-0008

FOOD ALLERGIES AND MISCONCEPTIONS ASSOCIATED WITH ITS DIAGNOSTIC WORK-UP AND ISSUES IN MANAGEMENTS

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ABSTRACT

Diagnosing food allergies can be as complicated as the medical condition itself. Symptoms of food allergy can vary from person to person and a single individual may not always experience the same symptoms during every reaction. Food allergic reactions can affect the skin, respiratory tract, gastrointestinal tract, and/or cardiovascular system and people develop food allergies at various ages.

Diagnostic food allergy testing offers clues about the causes of symptoms but it cannot determine whether someone has a food allergy with absolute certainty without a challenged study. Still, when a food allergy is suspected it's critically important to consult with an allergist who can determine which food allergy tests to perform, determine if food allergy exists and counsel patients on food allergy management once the diagnosis has been established.

To make a diagnosis it is essential to take a detailed history and a relevant physical examination. It may be required to perform a skin prick test for food allergy or a blood test (such as an ImmunoCAP test) and/or tests to determine food-specific IgE antibodies.

Skin prick tests are conducted in a doctor's office and provide results within 15 - 30 minutes. A nurse or the allergist administers these tests on the patient's arm or back by pricking the skin with a sterile small probe that contains a tiny amount of the food allergen. The tests, which are not painful but can be uncomfortable, are considered positive if a wheal (resembling a mosquito bite bump) develops at the site.

The blood tests, which are less sensitive than skin prick tests, measure the amount of IgE antibody to the specific food(s) being tested. Results are typically available in about one to two weeks and are reported as a numerical value.

The allergist can interpret these results and use them to aid in a diagnosis. While both of these diagnostic tools can signal a food allergy neither is conclusive. A positive test result to a specific food does not always indicate that a patient will react to that food when it's eaten. A negative test is more helpful to rule out a food allergy. Neither test, by its level of IgE antibodies or the size of the wheal, necessarily predicts the severity of a food allergic reaction.

Together with the patient's history, an allergist may use these tests to make a food allergy diagnosis. In some cases, an allergist may wish to conduct a double-blinded, placebo-controlled oral food challenge which is considered to be the gold standard for food allergy diagnosis. However, the procedure can be costly, time-consuming and in some cases is potentially dangerous so it is not routinely performed.

During an oral food challenge, the patient is fed tiny amounts of the suspected allergy-causing food in increasing doses over a period of time under strict supervision by an allergist. Emergency medication and emergency equipment must be on hand during this procedure.

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SS-Imm-0009

BERGININ LOADED XANTHAN STABILIZED SILVER NANOPARTICLES PREVENT ARTHRITIS BY SUPPRESSING TH1/TH2 IMMUNE RESPONSE AND TLR2/TLR4 mRNA EXPRESSION IN ADJUVANT-INDUCED ARTHRITIS

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ABSTRACT

Objective: Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of joints associated with massive infiltration of activated immune cells and cartilage or bone destruction. Various plant-derived flavonoids and polyphenols have been found to be effective in controlling RA progression. Bergenin (BG) is a C-glucoside, hydrolysable tannin possesses various pharmacological activities and however, its stability & bioavailability are majorly compromised. The aim of the study is to demonstrate the successful fabrication of gum xanthan stabilized silver nanoparticles with bergenin (GX-AgNPs-BG) and further tested against CFA-induced arthritis model targeting ROS, cytokines & TLRs &Th17-associated transcription factor ROR-γt expressions.

Material and Methods: Gum xanthan stabilized silver nanoparticles with bergenin (GX-AgNPs-BG) production and its characterization were analyzed through zeta sizer, UV–vis, AFM and FT-IR. For the confirmation of its anti-arthritic activity, Complete Freund's adjuvant (CFA) (0.1 ml) was injected into the paw of female wistar rats to induce arthritis and treatment with BG (25 mg/kg; *p.o*.) & GX-AgNPs-BG (1 mg/kg*; p.o*.) was given up to 14 days, volume was determined by Vernier caliper. For the assessment of arthritic damage, histological analysis of knee joints was performed by using (H & E) stain and X-Ray. Expression of TLR2, 4 and ROR-γt was evaluated by RT-PCR. Moreover, IL-6, IL-1β and TNF-α pro-inflammatory cytokines and anti-inflammatory cytokines TGF-β1 and IL-10 were analyzed in serum of arthritic and treated rats. The levels of RANKL, OPG, IL-17, MMP9 and MMP2 cytokines were identified in synovial tissue. For production human neutrophils (1×10^4 neutrophils/mL) were preincubated (PMA) and (OZ) for 5 min at 37 °C, followed by the addition of BG and GX-AgNPs-BG (1-10 µg/mL) and absorbance was measured at 450 nm.

Results: The results demonstrated the successful fabrication of gum xanthan stabilized silver nanoparticles with bergenin (GX-AgNPs-BG) characterized through UV–vis, zetasizer, FT-IR and AFM. BG and GX-AgNPs-BG exhibited potent anti-arthritic activity with minimal arthritic score, mild to moderate paw tissue swelling, reduced degenerative changes along with mild articular changes and lesser influx of inflammatory cells in macroscopic, X-ray and histological examination, respectively. The treatment of BG and GX-AgNPs-BG significantly suppressed the levels of cytokine levels and to antagonize the oxidative stress *via* interference with NADPH oxidase metabolic pathway. The treatment also suppressed the levels of RANKL, OPG, IL-17, MMP9 and MMP2 cytokines in synovial tissue and also the TLR2, 4 and ROR-γt expression in spleen tissue. Moreover, increased production of $O_2^{\bullet-}$ in human neutrophils stimulated by opsonized zymosan (OZ) and phorbol-12-myristate-13-acetate (PMA) was also suppressed.

Conclusion: Taken together, the current investigation validated that GX encapsulated AgNPs as stable nano-cargo for embattled delivery of BG with improved stability, enhanced efficacy, increased solubility and targeted delivery meets the criteria for drug formulation and might be a new promising multi-targeted therapeutic strategy for RA treatment.

Key Words: Gum xanthan, Silver Nanoparticles, Bergenin, Arthritis, mRNA Expression

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SS-Imm-0010

EXPERIENCE OF PID AT A TERTIARY CARE CENTER IN KARACHI

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IMMUNOLOGY FREE PAPER ABSTRACTS

FP-Imm-0001

SCREENING OF LATENT TUBERCULOSIS AMONG HEALTH CARE WORKERS USING IGRA

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ABSTRACT

Objective: Pakistan ranks 6th amongst high TB burden countries. Annually around 430,000 people contract tuberculosis (TB) in Pakistan with~70,000 deaths attributed to the disease. Latent tuberculosis is asymptomatic and in-transmissible diseases. Health care workers dealing with TB samples and patients are always at high risk of TB infection. In this study we have investigated frequency of latent TB in health care workers working in laboratories and patients suspected to have been exposed to TB. To investigate healthy health care workers for latent TB using QuantiFERON Assay.

Study Design: Cross Sectional

Methodology: A total of 77 healthy healthcare workers and 104 patients diagnosed with TB were recruited in this study. 3ml of blood was collected and dispensed into three specific QFT tubes. Samples were investigated for latent TB by QuantiFERON TB Gold kit.

Results: Out of 77 healthy healthcare workers, 16.8% (n=13) were positive for latent TB. Among them 36.3% (n=4) were microbiologists, 25% (n=5) were technicians, 37% (n=3) were phlebotomists while 50% (n=1) of the housekeeping staff was also positive for Latent TB. However, none of the doctors and medical technologists was positive for latent TB. While out of 104 patients, 23.1 % (n=24) were positive, 10.5% (n=11) were indeterminate and 65.3% (n=68) were negative for LTB. Statistical analyses revealed 35.14% risk to exposed individual with the overall estimated risk was 42.54% with risk ratio of 0.79%.

Conclusion: This study reported high prevalence of LTB in HCWs and patients. Microbiologists and technicians were highly affected. Housekeeping staff was also positive with LTB. This study also estimated high risk to technicians, medical technologists, microbiologist and phlebotomist as compared to doctors, housekeeping staff and interns. The results recommended the regular screening of the HCWs to ensure their and others safety as no one is safe until everyone is safe.

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FP-Imm-0002

DISTRIBUTION OF HLA-B*27 SUBTYPES IN PATIENTS WITH ANKYLOSING SPONDYLITIS IN PAKISTAN

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ABSTRACT

Introduction: HLA-B*27 has more than a hundred alleles. Different alleles affect the structure as well as the charge of the peptide-binding groove of the HLA-B27 protein resulting in either susceptible alleles with an increased binding affinity of arthritogenic peptides or in protective alleles with reduced binding affinity.

Objective: The purpose of this study was to compare the distribution of HLA-B*27 subtypes in healthy controls and in AS patients from different ethnic groups from Pakistan.

Design: Case control study. *Place and duration:* Armed Forces Institute of Pathology from April 2016 to Oct 2017.

Patients and Method: Forty-nine HLA-B*27 positive, unrelated AS patients and 18 HLA-B*27 positive healthy BMT/renal transplant donors were selected for this study. Typing of the HLA-B27 alleles was performed by the polymerase chain reaction-sequence-specific primer (PCR-SSP).

Results: Our results show a wide number of HLA-B*27 subtypes and an elevated frequency of the B*2707 allele in the AS patients. The allele B*2706 seems to have a protective role in the population studied because it was found only in the healthy controls. HLA-B*27:03 and 07 were found to be the predominant subtypes in Punjabis and Pathans respectively.

Conclusion: There were no significant differences for the distribution of B*27 subtypes between patients and controls ($p > 0.05$).

Keywords: Autoimmunity, Ankylosing spondylitis, Human leukocyte antigen, Alleles, Polymorphism, Ethnic groups, PCR-SSP

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FP-Imm-0003

MOLECULAR CHARACTERIZATION OF ACNE VULGARIS

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ABSTRACT

Acne vulgaris is a chronic inflammatory disease of skin characterized by inflammed and non-inflammed lesions in the form of papule, pustule, nodule and cysts. Global incidence is 1 case per 947 individuals per year. Prevalence of acne in Pakistan is 1 case per 633 individuals per year. Inflammation plays a strong role in pathogenesis of acne and proinflammatory cytokines are key players in these inflammatory events. Single nucleotide

polymorphism in inflammatory cytokines like TNF alpha is considered to influence the pathogenesis of acne.

The current case control study was aimed to evaluate the potential role of TNF-alpha-863 (rs1800630) and -1031 (rs 1799964) in development of acne vulgaris in Pakistani population. Blood samples of 100 acne patients and 100 age and gender matched healthy controls were recruited from same ethnic group. Serum TNF and CRP levels were measured by Enzyme -linked Immunosorbent assay. TNF-alpha genotyping was performed by polymerase chain reaction (PCR) and restriction fregment length polymorphism (RFLP).

Elevated TNF-alpha was observed in acne patients vs. unaffected controls (P = 0.001). CRP levels were also high in patients compared to controls (P < 0.001). The genotypes of TNF-alpha -1031T>C showed marginal association when compared to the controls ($\chi2$= 5.71, P = 0.05). The frequency of heterozygous TC genotype in patients was higher than in controls. However, no significant association was found between the variant allele C and the disease. Moreover, significant association between TNF-alph-863 C>A polymorphism and acne vulgaris has been observed. The variant genotype CA+AA was more prevalent among the acne patients (P = 0.02, $\chi2$ = 4.62). Higher incidence of -863 A allele and CA+AA genotype was found in acne patients compared to healthy control indicating the increased risk of acne patients (OR = 0.5, 95%CI = 0.304-0.909, P = 0.02). Significant association of disease severity with TNF-alpha and CRP was found in patients (P = 0.001). Non-significant relationship of variant genotype at TNF-alpha-1031 and -863 and serum TNF-alpha and CRP levels were found in patients. It can be concluded that TNF-alpha rs1800630 might play a key role in acne formation in studied population.

Key Words: TNF, RFLP, ACNE Vulgaris

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FP-Imm-0004

THE ASSOCIATION AND CORRELATION OF GENETIC POLYMORPHISM OF THE INTERLEUKIN-10 (-819) GENE AND ITS RESPECTIVE CYTOKINES IL-10 IN SALIVA WITH PERIODONTAL DISEASE IN PAKISTANI POPULATION

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ABSTRACT

Objectives: Interleukin-10 (IL-10) is an anti-inflammatory cytokine that has multiple roles in the periodontal diseases. The polymorphism of IL-10 gene in the promotor region has been associated with various IL-10 expressions. The aim of this study was to assess the association of Interleukin-10 (-819) gene polymorphism and to analyze the levels of salivary anti-inflammatory cytokine IL-1O with chronic periodontitis in a Pakistani population.

Materials & Method: This case control study was conducted in Dow University of Health Sciences, Karachi, Pakistan. After clinical examination and consent, blood and saliva was collected as per protocol. Genomic DNA extracted from the blood was amplified using conventional PCR with specific primer flanking the locus -819 of IL-10. Sanger sequencing of PCR products in order to check the polymorphism was carried out, results were investigated with MEGA 7 software and Electropherogram were visually inspected to check the polymorphism and genotype were investigated using the reference sequence. Salivary cytokine levels of IL-10 were analyzed with enzyme-linked immunosorbent assay (ELISA). SPSS 17 was used for Data analysis. P-values < 0.05 was considered as significant.

Results: There were statistically significant differences between Cases and Controls regarding clinical parameters (P < 0.05). A significant difference was also observed in the allelic frequencies between the two groups showing high prevalence of T allele in healthy individuals and C alleles were significantly higher in Patients (Pearson $\chi2$= 12.16, P < 0.001). Homozygous genotype CC was found significantly higher in periodontitis patients whereas TT genotype showed a significant association with healthy individuals ($\chi2$= 8.247, P=0.016). Considerably lower levels of salivary cytokine IL-10 were observed in periodontitis subjects as compared to the healthy group and significant correlation have been observed with single nucleotide polymorphism of *IL-10 -819* and salivary cytokine levels.

Conclusion: Our study concludes that polymorphism of IL-10 -819 could be a risk factor for periodontitis in the Pakistani population.

Key Words: Periodontitis, Interleukin 10, Single Nucleotide Polymorphism, ELISA.

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FP-Imm-0005

CLINICOPATHOLOGICAL FEATURES OF ANTI NMDA RECEPTOR ANTIBODY ENCEPHALITIS IN OUR POPULATION

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ABSTRACT

Objective: To present clinicopathological features and treatment outcome of 8 patients with anti NMDA receptor antibody mediated encephalitis with a view to document evidence to facilitate timely diagnosis and recommend specific therapy.

Type of study: Case series

Duration: December 2016 to February 2018

Institution: Shifa International Hospital.

Inclusion criteria: Patients with anti NMDA receptor antibody Encephalitis.

Exclusion criteria: Patients with any other psychiatric illness.

Results: Eight patients tested positive out of a total of 247 specimens received with suspicion of anti NMDA receptor antibody encephalitis. There were 7 females and 1 male with a mean age of 15 years and age ranging from 1 year to 28 years at presentation. Prodrome with fever and flu like illness was observed in 5 patients, seizures in 8 patients, memory deficit in 4 patients, delusions and paranoia in 2 patients and hallucinations were documented in 1 patient. The youngest 3 patients presented with hyperactivity and irritability along with seizures. Five patients responded well to a combination of steroids and IVIg and showed complete recovery. One patient received steroids, followed by plasmapheresis and IVIg and recovered completely. Two patients were treated with steroids only and suffered residual speech and motor deficit.

Conclusion: Anti NMDA receptor antibody was found in about 3.2% of specimens referred for this test. Anti NMDA receptor antibody encephalitis should be suspected in young females presenting with seizures, memory deficit, delusions, paranoia, hallucinations and altered consciousness. Children present with hyperactivity, irritability, seizures and altered consciousness. Patients who are treated with combination immunosuppressive therapy including steroids, IVIg and plasmapheresis have a high probability of complete recovery.

Keywords: Anti NMDA receptor Encephalitis, Immunosuppressive therapy, steroids, plasmapheresis, IVIg.

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FP-Imm-0006

ANTI-ATHEROSCLEROTIC EFFECT OF OPUNTIOL AND ITS SILVER NANO PARTICLE AGAINST FOAM CELL FORMATION VIA NUCLEAR FACTOR KB SIGNALING

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ABSTRACT

Objectives: Atherosclerosis is an inflammatory disease, characterized by the accumulation of macrophage-derived foam cells in the vessel wall and accompanied by the production of a wide range of chemokines and cytokines predominantly regulated by Nuclear Factor κB Pathway.

Study Design: The present study addresses the anti-atherogenic and anti-inflammatory potential of opuntiol (OP) and its silver nanoparticle (OP-AgNPs) against oxidized low-density lipoprotein (ox-LDL)-induced macrophage foam cell formation and cytokine production including TNF-α, IL-1β and MCP-1.

Method: Murine peritoneal macrophages were collected after 72 hours of peritonitis and kept in overnight incubation. The supernatant was removed after 24hrs and the adherent macrophages further incubated with ox-LDL (50µg/ml) followed by treatment with OP (1, 5 & 10µg/ml) and OP-AgNPs (0.5, 1 &3µg/ml). After 24 hours of treatment, Oil Red O staining was performed to observe the inhibitory effect of treatment against foam cell formation under light microscope. mRNA expression of TNF-α, IL-1β and MCP-1 was determined through real-time PCR and NFkB expression was examined by ELISA in ox-LDL-induced macrophage foam cells.

Results: The nano-formulation was successfully characterized through Atomic force microscopy (AFM) and Dynamic light scattering (DLS) analysis. A total of 65 ±3.6% of murine macrophages cells displayed foamy characteristics with oil red staining of lipid droplets after incubation with ox-LDL for 24 h, however OP and OP-AgNPs treatment significantly reduced the foam cell formation in a dose-dependent manner. Additionally, OP and OP-AgNPs significantly inhibited the mRNA expression of ox-LDL-induced TNF-α (63-85%), IL-1β (55-78%) and MCP-1(59-83%) cytokine production indicating that the compound has potential to effect at transcriptional level. The treatment also significantly suppressed ox-LDL induced IκB-α degradation and nuclear p65 expressionin macrophages, indicating that opuntiol could inhibit the activation of NF-κB signaling during atherogenic phenomenon.

Conclusion: It can be concluded that opuntiol and opuntiol coated silver nanoparticle potentially interfered with ox-LDL-induced macrophage foam cell formation and cytokine production including TNF-α, IL-1β and MCP-1 *via* NF-κB signaling, thus can be considered for the development of novel anti-atherogenic agent to encounter the future challenges in cardiovascular ailments.

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FP-Imm-0007

ANTI- SACCHAROMYCES CEREVISIAE ANTIBODIES AND ANTI- NEUTROPHIL CYTOPLASM ANTIBODIES INFLAMMATORY BOWEL SYNDROME VERSUS IRRITABLE BOWEL DISEASE

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ABSTRACT

Objective: Inflammatory bowel disease (IBD) and inflammatory bowel syndrome (IBS) are very distressing gastrointestinal disorders with overlapping symptoms. The prevalence of IBS is around 13% in Pakistani population while prevalence of IBD has wide variation due to its

heterogeneous manifestation and delayed diagnosis. IBD comprises of two pathologically distinct entities including ulcerative colitis (UC) and Chron's disease (CD). Several immunological markers are now available to diagnose and distinguish between UC and CD including anti-Saccharomyces cerevisiae antibodies (ASCA)and perinuclear anti- neutrophil cytoplasmic antibodies (p-ANCA).

We aimed to study the sensitivity and specificity of ASCA and p-ANCA in patients presenting with features of IBS or IBD.

Study Design: It was a retrospective analytical study done in the department of Immunology at SIUT from January to September (2018).

Materials and Methods Laboratory data of 25 patients with gastrointestinal complaints was analyzed retrospectively for clinical features and the presence of ANCA and ASCA. The ASCA IgA and IgG antibodies, anti-MPO and anti-PR3 were detected by ELISA, while c- and p-ANCA were tested by indirect immunofluorescent assay using commercially available kits. LFTs, PT, APTT, blood CP and histopathology report were also noted.

Results: The mean age of the patients was 32.6±19 years with 14(56%) females. Of total 11 patients had positive ASCA and four had ANCA positivity. Ten patients had symptoms of IBS or IBD. Of these five patients were positive for ASCA while only one had atypical p-ANCA. The sensitivity and specificity of ASCA was found to be 50% and 60% respectively.

Conclusions: This study although comprises of a small sample size but provides useful information about the utility of immunological markers in IBD in our local population. ASCA with reasonable sensitivity and good specificity shows promising utility in IBD diagnosis. However, a prospective study on a large sample size is warranted.

Key words: Anti- Saccharomyces cerevisiae antibodies, Crohn's disease, Irritable bowel disease, Perinuclear anti-neutrophil cytoplasmic antibodies, Ulcerative colitis

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FP-Imm-0008

EVALUATION OF SERUM FREE LIGHT CHAIN ASSAY FOR DIAGNOSIS AND MONITORING OF PLASMA CELL DISORDERS

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ABSTRACT

Objective: To determine diagnostic accuracy of serum free light chain assay compared to serum and urine protein electrophoresis in plasma cell disorders.

Study design: Descriptive cross-sectional study

Methodology: Patients referred to AFIP for diagnosis of plasma cell disorders or for monitoring while on treatment were included in the study. They were tested for serum and urine protein electrophoresis (SPE and UPE) and immunofixation (IF) and serum free light chain assay(sFLC). Immunofixation was used as reference standard. Test results are compared in terms of sensitivity, specificity and accuracy.

Results: One hundred and three patients were tested for monoclonal gammopathies since May 2017 to May 2018. Sixty-four were newly diagnosed and 39 were for treatment monitoring. SPE had a sensitivity of 70.5% and specificity of 100%, sFLC had a sensitivity of 87% and specificity of 81%, UPE had a sensitivity of 23.5% and specificity of 97%. Accuracy index was 80.5% for SPE, 85% for sFLC and 54% for UPE. When taken together SPE and UPE had a combined sensitivity of 72% and specificity of 97% with accuracy index of 80.5%. SPE and sFLC had combined sensitivity of 98.5% and specificity of 84.3% with accuracy index of 96%.

Conclusion: Combination of SPE and sFLC has the highest sensitivity and accuracy for diagnosis and monitoring of plasma cell disorders compared to conventional testing with SPE and UPE.

Key words: SPE: serum protein electrophoresis, sFLC: serum free light chain assay, UPE; urine protein electrophoresis

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FP-Imm-0009

TH1, TH2 & TH17 CYTOKINES AT THE BASELINE AS A PREDICTOR OF LONGTERM ORGAN DAMAGE: PRELIMINARY RESULTS OF A 7-YEAR PAKISTANI SLE COHORT

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ABSTRACT

Objectives: To evaluate the value of TH1, TH2 & TH17 cytokine concentrations in Pakistani patients with SLE at the time of first diagnosis in predicting long term organ damage.

Study Design: Cross sectional study at the outset followed by long term follow up.

Methodology: The patients were initially screened for induction in the study at the Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi from June 2010 to December 2012. The patients were followed up at Combined Military Hospital Lahore for subsequent evaluation from April to September 2018.30 SLE patients were included in the study on fulfilling revised ACR criteria (1997) at the time of first diagnosis. A liquid microbead-array based cytokine profile evaluation was carried out on Luminex 200® analyzer to evaluate serum levels of IL-2, IL-4, IL-6, IL-8, IL-10, GM-CSF, IFN-gamma, and TNF-alpha. The patients were followed up over a 7-year period and re-evaluated for organ damage by calculating SLICC SLE damage index.

Results: A higher percentage of SLE patients presented with elevated IL-8 & IL-10 concentrations in their sera at the baseline. However, at a mean follow-up of 7 yrs and 3 months, elevated IFN-gamma & IL-10 levels at the time of initial diagnosis proved to be a better predictor of long-term organ damage.

Conclusion: Elevated IFN-gamma and IL-10 concentrations in Pakistani SLE patients at the time of initial diagnosis serve as a good predictor of long-term organ damage.

Keywords: *SLE, cytokines, SLICC score.*

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FP-Imm-0010

AUTOSOMAL RECESSIVE AGAMMAGLOBULINEMIA - FIRST CASE WITH A NOVEL TCF3 MUTATION FROM PAKISTAN

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ABSTRACT

Objective: Autosomal Recessive Agammaglobulin-emia (ARA) is an uncommon type of primary immunodeficiency characterized by mutations in genes responsible for early B cell differentiation and function. One such gene is the TCF3 gene, which encodes a transcription factor important for immunoglobulin gene expression. We present the case of a 9-year-old girl with history of diarrhea and recurrent pneumonias. Genetic analysis identified a TCF3 gene base deletion covering exons 5-11

Material & Methods: A 9 years old daughter of consanguineous parents presented with a history of chronic diarrhea and recurrent pneumonias since one year of age. She had received both inactivated and live vaccines without sequelae. No other family members had a history suggestive of a primary immunodeficiency. Her physical examination was notable for signs of anemia. Her height and weight were found to be <fiftieth percentile. Lymph nodes and tonsils were normal in size. Her investigations confirmed the presence of anemia, absent CD19+ B cells, and pan-hypogammaglobulinemia. The patient improved on antibiotics and intravenous immunoglobulin replacement.

Results: Targeted next-generation sequencing revealed a large deletion spanning exons 5 through 11 in TCF3. These exons encompass 238 of the protein's 652 amino acids, and encode the region between the two activation domains, as well as part of the second activation domain.

Conclusion: To our knowledge, this is the second case report of a homozygous mutation in TCF3. Immunoglobulin replacement therapy and antimicrobials are the mainstay of treatment for agammaglobulinemia with absent B cells. However, these modalities are not curative and there is an ongoing struggle to come up with new treatment modalities to cure this primary immunodeficiency.

Key Words: TCF3 gene, Autosomal Recessive Agammaglobulinemia, Primary immunodeficiency.

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FP-Imm-0011

CLINICAL CHARACTERISTICS OF PATIENTS WITH GLOMERULONEPHRITIS AND LOW FACTOR H

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ABSTRACT

Objective: Activation of the complement system is a key step in the protective innate immune response against injury, although it may also cause harm if left unchecked. The soluble complement inhibitor factor H (FH) exert a tight regulation on alternative pathways of complement activation, consequently, avoiding serious damage to host tissues. Low FH levels may be due to increased consumption and or decrease synthesis and manifest clinically as atypical hemolytic uraemic syndrome (aHUS), membranoproliferative glomerulonephritis (MPGN) type II and age-related macular degeneration.

We aimed to study the clinical characteristics and outcome of patients with low FH level presenting with renal involvement.

Study design: Retrospective clinical and laboratory data analysis.

Place of Study: Department of Immunology, SIUT.

Material and Methods: A total number of 170 tests were performed to detect FH levels between August 2015 to August 2018, out of which 19 cases had low FH levels. Of these complete clinical records and laboratory data of eight patients was available and analyzed.

The factor H level was detected by ELISA using commercially available Hycult biotech kit. (Reference range: 250-600μg/ml)

Results: The mean age of the patients with low FH was 10.6 ± 2.4 years with6(75%) males. The mean level of FH was 52.93 ± 47.8 μg/ml. The most common presenting complain was acute renal failure, fever was present in only two cases and hematuria in one. Of total 4 patients had diagnosis of aHUS both clinically and histopathologically. Three patients had only clinical diagnosis of aHUS with one also had G6PD deficiency. One patient had MPGN. With treatment six patients improved while two developed end stage renal disease.

Conclusions: Factor H is an important test for diagnosis of aHUS. If diagnosed and managed early most of these patients have good prognosis.

Key Words: Factor H, Atypical Hemolytic Uraemic syndrome, Glomerulonephritis.

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FP-Imm-0012

SUPPRESSOR OF CYTOKINE SIGNALING (SOCS)-3 DOWN REGULATION IS ASSOCIATED WITH INCREASED PROINFLAMMATORY RESPONSES IN DIABETIC INDIVIDUALS WITH *M. TUBERCULOSIS* INFECTION

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ABSTRACT

Objectives: Pakistan ranks 5th in high tuberculosis (TB) burden countries and 6th in countries with high burden of diabetes mellitus (DM). Individuals with diabetes have been shown to exhibit dysregulation of host protective cytokines such as IFN-γ, TNFα and IL6. Suppressor of cytokine signaling (SOCS) molecule 3 plays a role in maintaining balance between pro and anti-inflammatory cytokines in patients with TB. Given the co-incidence of both diseases we investigated the role of SOCS3 in modulation of host immunity in individuals infected with the pathogen *Mycobacterium tuberculosis* (MTB), comparing latent (LTB) and active disease.

Study design: cross sectional

Material and Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from healthy controls (EC, n=20), those with LTB (n=10), DM (n=13), DM with LTB (n=15) and TB patients (n=15). PBMCs were stimulated with PPD (10µg/ml) for 18 hours. Cell supernatants were collected and tested for Th1/Th2 Cytokines. RNA was extracted from stimulated PBMCs and mRNA used for RT-PCR based analysis of IFNγ, TNFα, IL6 and SOCS3 genes.

Results: Measurement of stimulated cell supernatants showed increased levels of IFN-γ in DM-LTB as compared with EC (p=0.01), LTB (p=0.03), DM (p=0.01) and TB (p=0.03) cases. IL12 and TNFα levels were increased in DM-LTB as compared with LTB (p value: IL12, 0.005; TNFα, 0.006), DM (IL12, p=0.021; TNFa, 0.019). Levels of IL6 were increased in DM-LTB as compared with EC (p=0.001) and LTB (0.021). Coordinately, we observed increased IFN-γ (p=0.033) and IL6 (p=0.01) mRNA expression in patients with DM-LTB as compared with EC; also, increased TNFα mRNA expression as compared with LTB (p=0.034) cases. SOCS3 mRNA expression was decreased in DM-LTB as compared with LTB. In patients with TB, IFN-γ mRNA levels were increased as compared with EC (p=0.0457), while SOCS3 mRNA levels were decreased as compared with LTB (p=0.0188).

Conclusion: We observed increased pro-inflammatory response in diabetics with latent TB to be associated with a downregulation in SOCS3 expression. As SOCS3 is required for mycobacterial clearance, our data suggests that this could be a mechanism which increases susceptibility to active TB in DM cases who are infected with MTB.

Key Words: Tuberculosis, Diabetes mellitus, Latent TB.

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FP-Imm-0013

FREQUENCY OF ALLERGIC ASTHMA AND COMMON AEROALLERGENS SENSITIZATION IN PAKISTANI PATIENTS OF BRONCHIAL ASTHMA

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ABSTRACT

Objective: To determine the frequency of allergic asthma and 18 common causative aeroallergens sensitization among patients of bronchial asthma.

Study design: Descriptive cross-sectional study

Material & Methods: This descriptive cross-sectional study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from March 2014 to March 2016, and comprised clinically-diagnosed adult patients of bronchial asthma referred from various hospitals of Punjab and Sindh. Detailed history of each patient was recorded. Serum total immunoglobulin E level was determined using enzyme-linked immunosorbent assay. Skin prick test for 18 common aeroallergens was performed. SPSS 20 was used for data analysis.

Results: Of the 105 patients, 62 (59.05%) were males and 43 (40.95%) were females. The overall mean age for males and females was 29.9±10.2 years and 28.7±7.0 years respectively. Overall mean serum total immunoglobulin E was 285.01±241.39IU/ml. Frequency of atopy/allergic asthma was 59(56.2%) and patients with raised total immunoglobulin E had more chance of developing allergic asthma than those having normal immunoglobulin E ($p<0.05$). Frequency of allergen sensitization was the highest with house dust mite 35 (33.3%), followed by paper mulberry 33 (31.4%) and grass 28(26.7%) cases.

Conclusion: Prevalence of allergic asthma was high and the most common allergen causing highest sensitization was house dust mite.

Key Words: *Aeroallergens, Allergic asthma, Bronchial asthma, Atopy, Allergen sensitization.*

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FP-Imm-0014

CUMIN PROTEINS SUPPRESS CYTOKINES AND TLRS EXPRESSION IN INFLAMMATORY MODELS

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ABSTRACT

Objective: *Cumin* is used in traditional medicine for its antimicrobial, anticancer, and anti-inflammatory properties. The present work aimed to evaluate the anti-inflammatory and anti-arthritic activity of protein fraction partially purified from cumin.

Study Design: Isolation and purification of fractions of cumin to evaluate their potential as anti-inflammatory

activity using in-vivo models. Female albino mice weighing (20–30g) were obtained for carrageenan induced peritonitis model of inflammation and Female Wistar rats (150–200 g) were obtained for adjuvant induced arthritis model.

Materials and Method: The fractions containing low molecular weight proteins were obtained by ammonium sulfate precipitation and size exclusion chromatography. The separation profile was determined by SDS-PAGE. Incidence of arthritis after Freund's complete adjuvant injection was assessed macroscopically by examining paw swelling and redness on alternate days. Additionally, severity of arthritis was graded on the basis of arthritic score. Histological analysis of knee joints was performed by using (H & E) stain for the assessment of arthritic damage. Expression of TLR2 and TLR-4 were evaluated by RT-PCR. Further the effect of protein on Carrageenan-induced IL1β, TNF-α and MCP-1 was measured in peritoneal leukocytes by RT-PCR.

Results: Evaluation of anti-inflammatory activity showed reduction in neutrophil migration in carrageenan induce peritonitis model. Treatment also reduced the mRNA expression of pro-inflammatory cytokines such as IL-1β, TNF-α and MCP-1. The protein fraction also showed significant reduction in paw edema in complete Freund's adjuvant (CFA)-induced arthritis model. Estimation of suppression of mRNA level of TLR-2 and TLR-4 by real time PCR confirm their anti-arthritic role. Further histopathological studies of soft/bone tissues of ankle joint of rats revealed mild inflammation in arthritic model after treatment with cumin protein compared to standard drugs.

Conclusion: The study indicated that these activities could contribute significantly to the pharmacological properties of the cumin proteins.

Key Words: Cumin, Arthritis, Inflammation.

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FP-Imm-0015

A MECHANISTIC STUDY OF AEGICERASCORNICULATUM: ENOS, INOSAND NADPH SIGNALING PATHWAYS

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ABSTRACT

Objective: *Aegicerascorniculatum* (Linn.) Blanco (Family Aegiceritaceae) is a mangrove plant that has been used in folklore medicine and traditional healer for decades against asthma, diabetes, inflammation and rheumatism. Its chemical constituents include various phenols and polyphenols (flavonoids, triterpenes/saponins). Therefore, considering the folklore use, we have evaluated the anti-inflammatory and anti-oxidant effect of *A. corniculatum* extract in *in-vitro* systems with their underlying possible mechanism of action targeting iNOS and eNOS expressions in cell lines and production of O2•− in human neutrophils stimulated by phorbol-12-myristate-13-acetate (PMA) and/or opsonized zymosan (OZ).

Materials & Method: In the present investigation, anti-inflammatory and free radical scavenging effect of *A. corniculatum* stems extracts was evaluated by *in-vitro* models. For inducible nitric oxide synthase (iNOS) mRNA expression, murine macrophage cell line RAW 264.7 were stimulated with lipopolysaccharide (LPS) in the presence and absence of methanol and ethyl acetate extracts (5-20 µg/ml) for 5 hours and analyzed by using real time PCR. For endothelial NOS (eNOS)mRNA expression in human umbilical vein endothelial cell line were also analyzed by using real time PCR. For O2•− production human neutrophils (1×10^4 neutrophils/mL) were preincubated with (PMA) and (OZ) for 5 min at 37 °C, followed by the addition of methanol and ethyl acetate extracts (1–25µg/mL) and absorbance was measured at 450 nm.

Results: It is quite interesting, that methanol and ethyl acetate extract significantly down-regulated the mRNA expression of iNOS in LPS-induced murine macrophages and also the eNOS expression. While in both the systems (PMA and OZ), methanol and ethyl acetate extracts (1–10µg/mL) exhibited concentration dependent inhibitory effect on O2•− generation. However, methanol extract was only effective in neutrophils stimulated by OZ but failed to produce significant change in PMA-induced O2•− generation.

Conclusion: In this study, ethyl acetate and methanol extracts derived from *Aegicerascorniculatum* (stems) demonstrated pronounced anti-inflammatory and antioxidant potential. These extracts contain a plethora of chemicals that can scavenge free radicals, diminishes the respiratory burst and also exert a protective effect against oxidative damage via interference with NADPH oxidase metabolic pathway.

Key Words: *Aegicerascorniculatum,* mRNA, NADPH oxidase.

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FP-Imm-0016

ANALYSIS OF INTERFERON GAMMA (+874) POLYMORPHISM, ITS GENE EXPRESSION AND SERUM LEVEL IN PATIENTS OF *MYCOBACTERIUM TUBERCULOSIS* INFECTION

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ABSTRACT

Objective

- To determine the polymorphism of IFN-**γ** gene +874 in TB patients and healthy controls
- To determine the relative expression analysis of IFN-**γ** genein TB patients as compared to healthy controls
- To determine serum level of interferon gamma IFN-**γ** in TB patients and healthy controls

Study design: It was a case control study

Material and Methods: Ninety subjects were recruited for each of the two study groups. Five ml of EDTA blood from patients of TB and healthy controls was collected and processed for the detection of gene polymorphism of IFN-γ by sequence specific PCR after DNA extraction. Gene expression analysis was performed with real time PCR by using gene specific primers. Serum level of IFN-γ was determined by ELISA technique.

Results: Polymorphism of IFN-γ in two groups were analyzed, odd ratio at 95%CI for AA genotype was 2.447 and for AT genotype was 0.778 and for TT genotype was 0.542 (*p* value for AA genotype= 0.008). These results indicate that a statistically significant association was present between polymorphic AA genotype of IFN-γ and TB. Gene Expression of IFN-γ was decreased in TB more with polymorphic AA as compared to AT and TT genotypes as well as protein level was reduced in patients of TB.

Conclusion: It is concluded that IFN-γ gene (+874A/T) polymorphism is found significantly associated with TB infection in Pakistani population and expression of IFN-γ decreases in patients with polymorphic genotype AA at RNA as well as protein level.

Keywords: Interferon-γ, Mycobacterium tuberculosis, Single nucleotide Polymorphism

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IMMUNOLOGY POSTER PRESENTATION ABSTRACTS

P-Imm-0001

ANTI-INFLAMMATORY ACTIVITY AND MECHANISM OF ACTION OF DAONIL (SULFONYLUREA) IN CARRAGEENAN-INDUCED INFLAMMATORY MODELS

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ABSTRACT

Objective: The aim of present study is to determine the anti-inflammatory potential of Daonil, a sulphonamide class of anti-diabetic drug (sulfonylurea) on neutrophil migration after inflammatory stimulus and investigate the underlying molecular mechanisms mainly focusing on COX-2 inhibition.

Study design: We examined the effect of Daonil (sulfonylurea) on COX-2 pathway, as docking studies showed association of sulfonylurea with COX-2. Different levels of inflammatory cytokines were evaluated in two experimental animal models of inflammation developed by induction of carrageenan including paw edema in rats and peritonitis in mice.

Material & Methods: Wister albino rats (150-200g) and BALB/c mice (18- 20g) were obtained for paw edema and peritonitis model, respectively. Daonil was administered orally at a dose of 100, 200, and 300 mg/kg prior to carrageenan induced inflammation. In paw edema rat model, paw thickness below the ankle joint was measured before and 1, 2, 3, 4, and 5 h after the injection of carrageenan (0.3%) using digital Vernier caliper. The percentage inhibition of inflammation was calculated. While, in peritonitis mice model, after 5 hours of carrageenan induction (500ug/ cavity), mice were euthanized, and cells were harvested from the peritoneal cavity with 5 ml PBS. Aliquots of the peritoneal washes were used to determine total cell counts. Expression of COX-1, COX-2, TNF- α, and IL- 1β was analysed by RT-PCR and PGE2 was quantified.

Results: Oral administration of Daonil (sulfonylurea) at a dose of 100, 200, and 300 mg/kg, inhibits neutrophils infiltration (60-70%), COX levels (80-90%), and Prostaglandin E2 (PGE 2) (40-50%). While it decreases the level of inflammatory cytokines (TNF- α 70-90% and IL-1β~50%) comparable to anti-inflammatory drug Brufen (propionic acid) in carrageenan induced animal model.

Conclusion: The anti-inflammatory effect induced by Daonil (sulfonylurea) is due to inhibition of COX-2 and decrease production of inflammatory cytokines at the site of inflammation. Thus, reviving the secretion of insulin by protecting β-cells destruction in the same manner as anti-inflammatory drug.

Key Words: Inflammation, anti-diabetic, sulfonylurea, COX-2, cytokines

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P-Imm-0002

LC-MS/MS PROTEIN PROFILING OF SOLANUM NIGRUM AND EVALUATION OF IMMUNO-MODULATORY POTENTIALS IN INFLAMMATORY MODELS

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ABSTRACT

Objective: Proteomic analysis of *Solanum nigrum* and purification of protein with anti-inflammatory activity.

Study Design: In the present study, the proteome of *Solanum nigrum* was analyzed by shotgun proteomic approach. Functional characterization of biologically active protein leads to the purification of low molecular protein with anti-inflammatory activity.

Methodology: For proteomic analysis, proteins were separated 1D-gel electrophoresis. Peptides obtained after in-gel digestion were analyzed by state-of-the-art mass spectrometer. The 9 kDa protein was purified by combination of size exclusion and high-performance chromatography. In vivo studies were carried out to determined the suppression of the proinflammatory cytokines and mediators by purified protein as a characteristic of immuno-modulatory activity. As first step, carrageenan induced peritonitis models were used, leucocytes were counted and mRNA expression level of cytokines/chemokines were quantified by Real-Time PCR. In second step, Complete Freund's Adjuvant induced arthritic model was used to determine the arthritic score along with suppression of mRNA levels of TLR2 and TLR4. In last, histophotological analysis of soft and bone tissues of ankle joint was done after H&E staining.

Results: Proteomic analysis revealed significant knowledge of protein composition along with identification of several proteins with functional significance in various biological processes. Furthermore, anti-inflammatory and anti-arthritis activity of 9kDa protein was evaluated. The study showed, that protein treatment caused significant decrease in leukocyte infiltration in carrageenan-induced peritonitis in rats along with down regulated mRNA expression of IL-1β, TNF-α, and MCP-1. Additionally, evaluation of anti-arthritic activity in complete Freund's adjuvant induced arthritic model, revealed significant reduction in paw edema via suppressing transcriptional expressions of TLR2 and TLR4 along with arthritic scores and histopathological parameters.

Conclusion: This is the first report describing protein profiling of *Solanum nigrum* by shotgun proteomic approach. The purification of 9kDa protein with potent immuno-modulatory activity provides a molecular basis for future research on potential pharmacologically active proteins of *Solanum nigrum*.

Key Words: *Solanum nigrum*, Proteomics, Anti-inflammatory activity, Immuno-modulatory activity

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P-Imm-0003

CLINICAL SIGNIFICANCE OF CDC, PRE-TRANSPLANT DONOR SPECIFIC ANTIBODY IN RENAL TRANSPLANTATION: A CASE REPORT AT AGA KHAN UNIVERSITY HOSPITAL, KARACHI

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ABSTRACT

Objective: This case report investigates the value of pre-transplant panel of reactive antibodies (PRA) level on rejection and graft survival in patients after kidney transplantation.

Introduction: Pre-formed anti-human leucocyte antigen (HLA) antibodies may have a negative effect on a kidney transplant and result in graft rejection. Methods to test for antibody-mediated reactions between donor and recipient include, Complement Dependent Cytotoxicity (CDC) crossmatch and virtual cross match assays through integrated by solid-phase HLA-antigen coated bead methods (Luminex). Highly sensitized patients with PRA greater than 85% have a positive crossmatch which leads to graft rejection post-transplant. The degree of HLA mismatch between donor and recipient plays an important role in determining the risk of chronic rejection and graft loss. Identification of PRA in donor sera can be matched against the HLA type of the potential recipient to determine potential donor-specific antibodies (DSA).

Case: A 37-year-old female patient with known case of chronic kidney disease and hemodialysis dependent with negative autoimmune profile was worked up at AKUH for a potential kidney transplant. CDC crossmatch was done between patient and donor with whom she had a complete HLA Class I and II type match negative.

Materials and Methods: All samples were tested in parallel using the CDC and Luminex techniques (One Lambda technologies, USA).

Results: PRA pre-transplant tested by using PRA mixed assay revealed positive Class I and Class II antibodies. This was determined at 8% for SBA Class I and 20% for SBA Class II. The patient was subsequently desensitized and then retested after 6 months. No DSA was detected. The patient underwent kidney transplant successfully.

Conclusion: Determination of PRA prior to transplant is an important tool to identify potential graft rejection in transplant. A negative crossmatch and negative DSA gives a good indication of success in transplants.

Keywords: PRA (Panel Reactive Antibody), CDC (Complement Dependent Cytotoxicity); Donor specific Antibody (DSA), HLA (Human Leukocyte Antigen).

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P-Imm-0004

CD14 GENE (-159 C>T) POLYMORPHISM AND ITS SURFACE EXPRESSION ON MONOCYTES IN PULMONARY TUBERCULOSIS PATIENTS

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ABSTRACT

Objective: The study was designed to compare *CD14* (-159 C>T) gene polymorphism and its surface expression on monocytes among active pulmonary TB patients, TB patients during anti-TB treatment and healthy controls.

Study design: The study was a cross-sectional comparative study that was carried out in the Department of immunology, UHS Lahore and Gulab Devi Chest Hospital Lahore after the approval of Ethical Review Committee and Advanced Studies & Research Board of UHS.

Methodology: The study population comprised of three groups (pulmonary TB patients before treatment, pulmonary TB patients during treatment and healthy controls) whereas 53 blood samples were collected from each group from Gulab Devi Chest Hospital Lahore. The percentage of monocytes and CD14 mean fluorescence intensity (MFI) was measured by flow cytometry whereas PCR-RFLP was used to determine gene polymorphism.

Results: The results indicated that the percentage of monocytes did not change with the disease status whereas CD14 MFI was significantly high in healthy controls than in TB patients (p<0.0001). *CD14* SNP alleles (C, T) and genotypes (CC, CT, TT) had no significant difference between TB patients and healthy controls.

Conclusion: The study concluded that *CD14* gene (-159 C>T) polymorphism is not associated with pulmonary TB disease in a sample of Pakistani population and surface expression of CD14 receptor is decreased on peripheral blood monocytes in active TB disease.

Key words: Tuberculosis, CD14, Monocytes, Gene polymorphism

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Imm-0005

ROLE OF TNF A, IL-6 AND CXCL10 IN DENGUE DISEASE SEVERITY

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ABSTRACT

Objectives: Dengue virus infections (Dengue) have become increasingly common in Pakistan and can result in case fatalities if not managed appropriately. Patients with Dengue virus infection may be asymptomatic or present with Dengue fever (DF), Dengue with warning signs (DWS) or severe Dengue (SD). Severity in Dengue is coincident with an exacerbated production of lymphocyte-induced cytokines and chemokines which are associated with

plasma leakage. We investigated the association of circulating levels of cytokines such as Interleukin (IL)-6, tumor necrosis factor (TNF)-alpha and CXCL-10 in Dengue patients with differing severity of disease.

Study design: Cross sectional

Methodology: Dengue infection was confirmed by testing for human IgM to the Dengue virus. Dengue patients (n=58) and healthy controls (n=33) were recruited. Dengue patients were grouped into those with DF (n=39), DWS (n=15) and SD (n=4). Serum IL-6, TNFα and CXCL10 levels were tested by ELISA. The Mann Whitney U test was used for statistical analysis.

Results: Circulating levels of TNFα (p≤0.001) and CXCL10 (p≤0.001) levels were increased in Dengue patients as compared with controls. When patients were stratified for disease severity, it was observed that CXCL10 was increased in DWS as compared to DF (p=0.046). IL-6 levels were increased in patients with SD as compared to those with DWS (p=0.044). TNFα levels were not found to differ between different groups of Dengue patients.

Conclusion: Raised CXCL10 and TNFα levels were associated with increased clinical severity of Dengue infection and probably increased disease progression due to excessive inflammation and increased vascular changes in the patients.

Key Words: Dengue, Disease severity, Inflammation.

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P-Imm-0006

COMPARISON OF ANTI-CARDIOLIPIN & ANTI-BETA-2 GLYCOPROTEIN I ANTIBODIES IN PATIENTS WITH AND WITHOUT SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

Objective: The antiphospholipid antibody syndrome (APS) is a multisystem disorder characterized by recurrent thrombosis and abortions with laboratory evidence of antiphospholipid antibodies (APLA). APS is classified as primary or secondary to other autoimmune disorders. In systemic lupus erythematous (SLE), the prevalence of APLA has been reported to be around 40% with APS in 25% patients. The important APLA include anti-beta 2 glycoprotein I (aB2GP1) and anti-cardiolipin antibodies (ACA). The prevalence of these two antibodies in SLE and APS patients is variable and is influenced by ethnicity and geographical area.

We aimed to find out the prevalence of aB2GP1 and ACA and their isotypes in SLE with or without APS and non SLE patients with suspicion of APS

Study Design: Retrospective laboratory data analysis

Material and Methods: Analysis of laboratory and clinical data of 75 patients was done from January 2018 to June 2018 in the Department of Immunology at Sindh Institute of Urology and Transplantation. The samples were tested for aB2GP1 and ACA screen and their isotypes using commercially available ELISA kits.

Results: The mean age was 29.5 ± 10 years with 62(83%) females and 13(17%) males. In 27 patients diagnosis of SLE was present according to 1997 ACR and 2012 SLICC criteria. APLA was positive in 46 (61%) patients with aB2GP1 in 41 (89%) and ACA in 21 (45.7%). In SLE, APLA was found in 17(63%) with aB2GP1 in 16 and ACA in nine. Of these, eight patients had both aB2GP1 and ACA. In non-SLE patients, 29 (60%) had APLA with aB2GP1 positivity in 25 and ACA in 12 and eight had both autoantibodies. The most important APS feature in all these patients was recurrent abortions present in 15 with aB2GP1 positivity in 9 and ACA in 5. In patients positive for aB2GP1, IgA was the most prevalent isotype found in 33 patients while in ACA positive patients IgM was the predominant isotype.

Conclusions: aB2GP1 is the most prevalent autoantibody found in patients with suspicion of APS and in SLE with predominantly IgA isotypes. However, both aB2GP1 and ACA with all isotypes are important tests for the diagnosis of APS.

Key Words: Anti-B2 Glycoprotein, Anti-cardiolipin Antibody, Anti-phospholipid Antibody, Antiphospholipid Syndrome, Systemic Lupus Erythematosus

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Imm-0007

PREVALENCE OF ANTI-NUCLEAR AND ANTI-dsDNA ANTIBODIES IN SAMPLES TESTED AT AFIP RAWALPINDI AND THEIR CORRELATION WITH AGE AND GENDER

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ABSTRACT

Objective: To find prevalence of ANA and anti-dsDNA antibodies in samples tested at AFIP Rawalpindi and their correlation with age and gender and positive and negative predictive values of ANA.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: AFIP Rawalpindi from July 2017 to Sep 2018.

Materials and Method: Retrospective data of all samples (autoimmunity suspected group) that were tested by indirect immunofluorescence (IIF) for ANA and dsDNA antibodies, was collected along with age and gender. To address positive and negative predictive values another group (autoimmunity not suspected) of serum samples was selected which were sent to AFIP for testing of serum total IgE levels. For first group, age, gender, ANA and dsANA results data was collected from computer record cell and for second group, ANA and dsDNA were performed by IIF.

Results: Total 12968 and 4704 samples (group 1) were tested for ANA and dsDNA antibodies respectively during this period. 902 (7%) and 98 (2%) were found positive for ANA and dsDNA antibodies and among these positive

samples, 627 (69%) and 70 (71%) were females respectively. Gender predisposition towards autoimmunity (ANA) and dsDNA was found significant with p value of <0.01 and <0.025 respectively. Age of ANA and dsDNA positive patients was ranging from 3 years to 87 years and 1 year to 82 year with mean age of 38 and 35 years respectively. Relation of mean age was also found significant with ANA and dsDNA with p value of <0.01 and <0.01 respectively. In second (autoimmunity free) group a total 468 samples were tested for ANA and 9 (2 %) were found positive. Positive predictive value was 99% and and negative predictive value was 3.7 %.

Conclusion: ANA is a sensitive test for autoimmunity and it is significantly related with female gender and increasing age. Low prevalence of ANA among clinically suspected cases suggests that rationalization of test prescription is needed.

Key Words: ANA, Anti dsDNA, autoimmunity, SLE, immunofluorescence.

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Imm-0008

PREVALENCE OF ANTI CCP ANTIBODIES IN CLINICALLY SUSPECTED CASES OF RHEUMATOID ARTHRITIS AND ITS RELATION WITH AGE AND GENDER

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ABSTRACT

Objective: To find prevalence of anti CCP antibodies in samples tested at AFIP Rawalpindi and its correlation with age and gender and positive and negative predictive values of anti CCP antibodies.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: AFIP Rawalpindi from July 2017 to Sep 2018.

Materials and Method: Retrospective data of all samples (RA suspected group) that were tested by ELISA for anti CCP antibodies, was collected along with age and gender. To address positive and negative predictive values another group (RA not suspected) of serum samples was selected which were sent to AFIP for testing of serum total IgE levels. For first group, age, gender and anti CCP antibodies results data was collected from computer record cell and for second group, anti CCP antibodies were performed by ELISA.

Results: Total 10194 samples (group 1) were tested for anti CCP antibodies during this period. 1978 (20%) were found positive and among these positive samples, 1290 (65%) were females. Gender predisposition towards autoimmunity (anti CCP antibodies) was found significant with p value of <0.01 and age of anti CCP antibodies positive patients was ranging from 2 to 98 years with mean age of 43 years. Relation of age was also found significant with anti CCP antibodies with p value of <0.01. In second (RA free) group a total 450 samples were tested for anti CCP antibodies and 16 (3.6%) were found positive. Positive predictive value was 99% and negative predictive value was 5.6 %.

Conclusion: Anti CCP antibodies is a sensitive test for rheumatoid arthritis and it is significantly related with female gender and increasing age. Low prevalence of anti CCP antibodies among clinically suspected cases suggests that rationalization of test prescription is needed.

Key Words: Anti CCP antibodies, Autoimmunity, rheumatoid arthritis, ELISA

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Imm-0009

EVALUATION OF SERUM IgE AND IVT SCREENING IN GENERAL POPULATION OF PAKISTAN

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ABSTRACT

Objective:

- The main objective of this study was to evaluate the elevated level of serum IgE and to find positive patients against different allergens.
- To create awareness in general population about pollution and sanitary conditions.

Material & Method: A cross-sectional study was conducted to evaluate serum IgE level in the population who visited our laboratory during last six months (i.e. from January 2018 to June 2018). A total of 510 serum samples were collected for this study. First, serum IgE level was detected by using Enzyme Linked Immunosorbent Assay (ELISA) technique. Further confirmation of allergy was done by in vitro testing of allergy using western blot method.

Study Design: Cross-Sectional study

Sampling Technique: Convenient sampling

Results: 280 samples out of 510 were showing elevated level of serum IgE. In in vitro testing of allergy 276 samples were positive against different type of allergens. Dp/Df H. Dust was seemed to be the most common allergen. 140(51%) patients showed reaction against dust. 110(39.8%) patients showed reaction against cow milk, 26(9.4%) samples showed allergic reaction against egg yolk/ white. Majority of samples found allergic from both dust and cow milk. Some showed low to moderate reaction against ragweed, cockroaches, mugwort & grass mix. It is found that population between the age group 15 to 30 years are the most affected one followed by age group 0 to 15 years.

Conclusion: Population becomes allergic to various allergens which is potentially harmful. Majority of environmental factors are playing an important role in getting these allergies. Due to poor sanitary conditions and the use of unhygienic food and water allergens are becoming more potent and harmful.

Key words: In-vitro, Immunoglobulin, Allergy.

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VIROLOGY SCIENTIFIC SESSION ABSTRACTS

SS-Viro-0001

RESPIRATORY VIRUSES

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ABSTRACT

The medical writings of Medieval India in Persian mention about many epidemics with high mortality which remind of Influenza pandemics of the past. The WHO Global Influenza Surveillance and Response System (GISRS) laboratories tested more than 68731 specimens during that time period from 03 September 2018 to 16 September 2018, 2512 were positive for influenza viruses, of which 2120 (84.4%) were typed as influenza A and 392 (15.6%) as influenza B. Of the sub-typed influenza A viruses, 1104 (65.3%) were influenza A(H1N1)pdm09 and 586 (34.7%) were influenza A(H3N2). Of the characterized B viruses, 54 (55.1%) belonged to the B-Yamagata lineage and 44 (44.9%) to the B-Victoria lineage. The WHO Consultation and Information Meeting on the Composition of Influenza Virus Vaccines for Use in the 2019 Southern Hemisphere Influenza Season was held on 24-26 September 2018 in Atlanta, United States of America. Since 2012 and as of 5 May 2018, more than 2200 cases of laboratory confirmed Middle East respiratory syndrome (MERS) have been reported to WHO. The virus is circulating widely throughout the Arabian Peninsula, and, to date, the largest numbers of human cases have been reported by Saudi Arabia. The primary objectives of surveillance are to: 1. Detect early cases of MERS-CoV infection, clusters and any evidence of sustained human-to-human transmission; 2. Determine risk factors and the geographic risk area for infection with the virus.

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SS-Viro-0002

ADVANCES IN DIAGNOSTIC VIROLOGY

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ABSTRACT

Viral infectious diseases account for a number of outbreaks, epidemics and even pandemics of global concern almost every year. They draw a lot media attention and are a source of anxiety for people around the world because of the associated mortality. Spectrum of viral illnesses range from serious pandemics to highly contagious infections to common influenza episodes. The clinical prognosis often relies on early detection of the infectious agent. The newer diagnostic tools have made viral laboratory services relevant to the clinical decision making. Thus, effective identification of viral pathogens is needed to help prevent transmission, set up appropriate therapy, monitor response to treatment and lead to efficient disease management and control. The aim of this lecture is to outline some of the recent technological advances in viral identification, including polymerase chain reaction, mass spectrometry and next-generation sequencing, and how they are applied in the diagnosis and management of viral infections. These powerful tools combine rapidity and efficiency in detecting viral pathogens and have revolutionized the field of clinical diagnostics

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SS-Viro-0003

VIRAL INFECTIONS IN BONE MARROW TRANSPLANT PATIENTS

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ABSTRACT

Generally, post-transplant early infections are those that occur before day 100, and late infections usually occur ≥100 days post-transplantation. Reactivation of HSV infection can occur at any time after bone marrow transplant (BMT). Use of prophylactic acyclovir has been very effective at reducing the rate of HSV reactivation from 80% to less than 5% in HSV seropositive recipients. CMV disease, which is the leading cause of morbidity and mortality in BMT patients, has profoundly decreased due to prophylactic and pre-emptive use of ganciclovir. The highest risk is for CMV negative recipients, who have a CMV Positive donor. Two approaches currently exist for patients at risk for CMV disease. One is to administer ganciclovir prophylaxis to every patient at risk of CMV disease. The disadvantage of this approach is that all patients are treated, leading to unnecessary treatment, myelosuppression and an associated increased risk of fungal infections. The other approach is preemptive treatment, which is the preferred method and adopted in many transplant centres. Weekly surveillance with CMV-DNA PCR is performed in all at risk patients in this approach. Varicella infections usually occurs in the late post-transplantation period and prophylaxis is not recommended in this period. However, prevention should be attempted following exposure and VZIG should ideally be administered within 48-96 hours after exposure. Reimmunization is a requirement for BMT recipients, because they have no immunity to the common childhood infections. Immunizations should be done in the first and second-year post-transplantation. Routine yearly

vaccination with influenza vaccine is recommended in all transplant recipients.

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SS-Viro-0004

CHANGES IN EPIDEMIOLOGY OF HIV/AIDS

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ABSTRACT

HIV/AIDS is a global pandemic. As of 2016, approximately 36.7 million people are living with HIV globally In 2016, approximately half are men and half are women There were about 1.0 million deaths from AIDS in 2016, down from 1.9 million in 2005. South & South-East Asia region has about 2 billion people as of 2010 and over 30% of the global population, estimated as 4 million cases living with HIV, with about 250,000 deaths in 2010. Approximately 2.5 million of these cases are in India, where however the prevalence is only about 0.3% (somewhat higher than that found in Western and Central Europe or Canada). The pandemic is not homogeneous within regions, with some countries more afflicted than others. Even at the country level, there are wide variations in infection levels between different areas. The number of people infected with HIV continues to rise in most parts of the world, despite the implementation of prevention strategies, Sub-Saharan Africa being by far the worst-affected region, with an estimated 22.9 million at the end of 2010, 68% of the global total. South and South East Asia have an estimated 12% of the global total.

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VIROLOGY FREE PAPER ABSTRACTS

FP-Viro-0001

DECLINING TREND OF HCV GENOTYPE TESTING IN PAKISTAN

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ABSTRACT

Objective: The aim of the study was to find out reasons for declining trend of prescription of HCV genotype testing.

Place of Study: Department of Virology, Chughtai Lab, Lahore

Material and Methods: A questionnaire based cross sectional study was designed. Data was collected during the month of July and August, 2018 from doctors after informed consent, working at different positions in Medicine/Gastroenterology department of concerned institutions, across all over Pakistan. Data was analyzed by using SPSS.

Results: Among 381 doctors responded, 255(66.9%) are not currently prescribing HCV genotype test and 126(33.1%) are still prescribing the test. 32% of the doctors use the test as a part of pre-therapeutic assessment of HCV treatment. WHO guidelines for HCV treatment; are the most commonly used 155(40.6%) and 145(38.2%) using EASLD guidelines, as a second choice with only 60(15.75%) preferred using AASLD guidelines. Affordability of test is found to be the most common reason 151(59.2%) for not prescribing HCV genotyping, 38(14.9%) considered the availability of pan-genotypic therapy as a reason, while 19(7.4%) are not prescribing due to non-availability of test at Governmental institutions, while others responded with more than one reason.

Conclusion: Affordability is the most common reason for declining trend of HCV genotype prescription, with the availability of pangenotypic drugs for HCV treatment being the second most common reason. Although clinically indicated the clinicians are dispensing with the test. It is a compromise due to constraint of resources.

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FP-Viro-0002

AN ANALYSIS OF CONCORDANCE BETWEEN HCV CORE ANTIGEN AND HCV PCR

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ABSTRACT

Objective: To study correlation between viral load of HCV by Real time PCR and Core antigen value of HCV and define an antiviral treatment monitoring cut off value for HCV core antigen.

Study Design: Cross sectional Study

Place and Duration of Study: Department of Virology, Chughtai Lab, Lahore from 6th June 2017 to 9thMarch2018.

Material & Methods: Hundred positive plasma samples for HCV RNA, confirmed by Abbott Real Time were subjected to HCV Core antigen (Abbott Architect i2000) in order to study the correlation between these two parameters. We divided the samples in 3 categories according to their viral load; <2000 IU/ml, 2000-10,000 IU/ml and >10,000 IU/ml. The data was recorded in the form of tables and charts and was analyzed by using SPSS version 21.0.

Results & Discussions: A total 100 sample were analyzed having viral load between 12 IU/ml 100,000 IU/ml during this study. 68% cases were female and remaining 32% was male. The age of patients ranged from 12 years to 80 years with mean age of 45 years. The samples were randomly selected from different regions of Pakistan. 73% were positive for HCV core antigen. Our results showed that the HCV core antigen was concordant with HCV RNA by PCR when the viral load was above 2000 IU/ml. Below HCV RNA load of 2000 IU/ml the sensitivity of HCV core antigen is 94.95 % and specificity of 88.89%. The positive predictive value (PPV) of this assay was found to be 89.52% while the negative predictive value (NPV) was 94.62% (Fig 1). Statistical analysis showed the significance of study for all groups with p value less than 0.05. There was positive correlation between these two variables. Our findings suggest that for patients having viral load above 2,000 IU/ml HCV core antigen value can be used as marker for diagnosis and monitoring the antiviral therapy. At the end of anti-viral treatment Real Time PCR should be performed for the confirmation of viral clearance. The above findings suggest HCV core antigen is a reliable test for initial viremic testing but may not be a good marker for HCV viremia for on treatment and 12 weeks post antiviral response (SVR12), where HCV RNA by PCR still holds firm.

Conclusion: HCV core antigen is a useful test for testing viremia pretreatment. All patients undergoing HCV core antigen testing at SVR12 should have a repeat HCV core antigen 3-6 months later, or alternatively HCV RNA by PCR should be the recommendation at SVR12 stage. In patients having viral load above 2,000 IU/ml, the HCV core antigen value can be used as a marker for diagnosis and monitoring the antiviral therapy. At the end of anti-viral treatment, Real Time PCR should be performed for the confirmation of viral clearance.

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FP-Viro-0003

EVOLVING HIV EPIDEMIC WITH EMERGENCE OF DIVERSE HIV SUBTYPES IN KARACHI, PAKISTAN

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ABSTRACT

Objective: HIV-1 prevalence is at horizon of incline in general population because of people engaged in high-risk

practices such as drug abuse, sexual activities, shared needles, healthcare exposure, or through unsafe blood transfusion and transmission from mother to child are frequently being observed in Pakistan, which accounted Pakistan a low-resource country where increase in prevalence is observed among high risk groups. So, it is important to identify circulating HIV-1 strains in Pakistan for the better understanding of the origin of emerging HIV-1 subtypes, recombinant forms and their transmission dynamics. In this study, we analyzed HIV epidemic in Karachi using gene sequencing and bioinformatics tool.

Study Design: Cross Sectional

Material & Methods: Total 50 infected patients (drug naive) were recruited in this study. Whole blood samples were collected from all patients. After DNA extraction, all positive samples were subjected to nested PCR using specific primers. The amplified products were sequenced and compared to reference sequences available at the LOS Alamos HIV Data base. The sequences of HIV infected patients were aligned and phylogenetic trees was constructed via the Maximum Likelihood method using MEGA 7.0 software. From the results of the test, statistics analysis was done on SPSS 20.

Results: Our results indicated that 81.25% of all the sequences were clustering with subtype A1. However, we also observed that few of the sequences were clustering with subtype C (6.26%), G (6.25%) and emerging recombinant subtype CRF11 (6.25%). Our data for geographic linkages are closely clustering with reference sequences of African countries.

Conclusion: We observed that majority of the sequences were clustering with previously reported circulating subtype A1, C and G. Furthermore, we also detected emerging subtype CRF11, that had never been reported from Pakistan and my study is first to report this subtype from the region. Our results are alarming and suggesting that these circulating viruses and those newly generated recombinants may become predominant strains in Pakistan.

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FP-Viro-0004

MOLECULAR DETECTION OF HUMAN PAPILLOMA VIRUS INFECTION OF UTERINE CERVIX AMONG WOMEN OF REPEODUCTIVE AGE

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ABSTRACT

Objective: The objective of this study was to determine frequency of human papilloma virus infection of uterine cervix among women of reproductive age by polymerase chain reaction.

Study design and duration: It is a cross sectional study of one-year duration.

Material & Methods: 162 samples of exfoliated cervical cells were collected with cervical sampler; during gynecological examination. The tip containing cellular material was then placed into viral transport medium tube and stored at 4°C and then transported to Virology department of AFIP for real time PCR.

Results: The frequency of human papilloma virus infection of uterine cervix among women of reproductive age by polymerase chain reaction was 2.47%(n=4). Mean age of participants was 33 years. Mean age at 1st pregnancy was 23 years while mean number of children was 3. 90% of participants husband have one wife. 91% never used OCP while 5.5% were former and 4.9% were current users. 89% of participants never smoked, 9.2% were former smoker and 1.8% was current smokers. Among positive cases three patients have HR-HPV 16 and one has a rare HR-HPV 51/59 infection.

Conclusion: The frequency of human papilloma virus infection of uterine cervix is not very high among women of reproductive in our setup however, it was a single center study which may not reflect the frequency of the whole city as data from other hospitals. A nationwide study must be carried out for formulation of screening and vaccination strategies for future.

Key Words: Reproductive age group, Human papilloma virus infection, Uterine cervix, Polymerase chain reaction

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FP-Viro-0005

MOLECULAR EPIDEMIOLOGY OF INFLUENZA A (H1N1) PDM 09 DURING POST PANDEMIC PERIOD IN PAKISTAN

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ABSTRACT

Introduction: Pandemic influenza A (H1N1) virus was first detected in Pakistan during June 2009 and continued to circulate causing considerable morbidity and mortality. The purpose of this study was to characterize Influenza A (H1N1) pdm09 viruses circulating in the post pandemic period and their relevance with the current vaccine viruses.

Material & Methods: Respiratory specimens were collected with influenza-like illness and Severe Acute Respiratory Illness and tested by Real-Time PCR according to CDC protocol. Phylogenetic analysis of Haemagglutinin (HA) and neuraminidase (NA) genes was carried out comparing representative Pakistani isolates.

Results: During 2009 - 18, 2773 (13%) samples were positive for influenza A out of 18378. During the pandemic period (2009–10), Influenza A/ H1N1pdm 09 was the dominant strain with 366 (45%) of total influenza positives. In the post-pandemic period (2011–2018), a total of 1969 (71%) cases were positive Influenza A/ H1N1pdm 09 with co-circulation of different Influenza A subtypes. Overall, the Pakistan A(H1N1) pdm09 viruses grouped in two genetic clades. Influenza A(H1N1) pdm09 viruses only ascribed to Clade 7 during the pandemic period whereas viruses belong to clade 7 (2011) and clade 6B (2015-18) during the post-pandemic years. Amino acid analysis of the HA gene revealed mutations at positions S220T, I338V and P100S

specially associated with outbreaks in all the analyzed strains. Sequence analyses of post-pandemic A(H1N1) pdm09 viruses showed additional substitutions at antigenic sites; S179N,K180Q (SA), D185N, D239N (CA), S202T (SB) and at receptor binding sites; A13T, S200P when compared with pandemic period. Analysis of NA gene revealed outbreak markers; V106I among pandemic and N248D during post pandemic Pakistan viruses.

Conclusion: Influenza A(H1N1) pdm09 viruses from Pakistan clustered into two genetic clades, with co circulation of some variants. Certain key substitutions in the Receptor binding site and few changes indicative of virulence were also detected in post pandemic strains. Therefore, It is imperative to continue monitoring of the viruses for early identification of potential variants of high virulence or emergence of drug resistant.

FP-Viro-0006

MOLECULAR PREVALENCE OF TRIPLE INFECTION HEPATITIS B, C AND D VIRUSES IN SINDH PAKISTAN

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ABSTRACT

Objectives: The aim of this study was to found out the molecular prevalence of triple infection HBV, HBC and HDV in Sindh.

Study design: Cross Sectional Descriptive Study.

Place and duration of study: It was carried out at Diagnostic & Research Laboratory LUMHS Hyderabad from June 2017 to date.

Material and Methods/ Results: Whole Blood samples were collected in 5 ml vacutainer. Total 21816 samples were received from our laboratories/ collection centers located in 20 different cities of Sindh for detection of different Hepatitis viruses by PCR. Out of that 5335 samples were for HBV, 15366 samples for HCV and 1115 samples for HDV detection. Serum was used to extract DNA & RNA of HBV and HCV respectively by Abbott m2000sp and was amplified on m2000rt automated instrument using commercially available kits of Abbott, while for HDV extraction was done using Roche high pure vial nucleic acid kit & implication was done using light cycler mix kit (Roche) on instrument z480 (Roche). Out of the total 5335 HBV samples 4275 (80%) were positive and 1060 (20%) were negative, Out of the total 15366 HCV samples 8763 (57%) were positive and 6603 (43%) were negative, Out of the total 1115 HBD samples 594 (52.3%) were positive and 521 (46.7%) were negative.

Conclusion: It is concluded that the only way to prevent these triple infections is by educating the general population by massive awareness programs, extensive vaccination and other preventive measures to stop the spread of these alarming diseases in Sindh, Pakistan.

Key Words: HBV, HCV, HDV, PCR

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FP-Viro-0007

ASSOCIATION OF ELISA BASED SEROLOGICAL MARKERS NS1 ANTIGEN, IGM, IGG ANTIBODIES AND RT-PCR IN THE DIAGNOSIS OF DENGUE VIRUS INFECTION IN PATIENTS ATTENDING DISTRICT HEADQUARTER HOSPITAL RAWALPINDI

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ABSTRACT

Objective: To determine the association of ELISA based serological markers NS1 antigen, IgM, IgG antibodies and RT-PCR in the diagnosis of dengue virus infection.

Material & Methods: In this descriptive study 420 serum samples from patients with suspicion of dengue fever were tested for detection of dengue by NS1 antigen ELISA, IgG, IgM ELISA and RT-PCR for dengue was carried out in all NS1 antigen ELISA positive cases for confirmation of dengue. Platelet count was recorded in all 420 suspected cases.

Results: Results obtained were analyzed, 249 cases out of 420 samples were positive for either one of the three markers NS1, IgM, IgG: Male to Female ratio was 301 (71.66%): 119(28.33%) respectively. 202 (48.09%) samples were positive for NS1 only, 13 (3.09%) were positive for NS1 and IgG. 07 (1.66%) were NS1, IgM and IgG positive,16 (3.80%) were positive for IgG only ,11 (2.61%) were positive for NS1 and IgM whereas 171 (40.17%) samples were reported negative for NS1, IgM and IgG. RT-PCR was conducted on 233 NS1 positive cases out of which 188(80.06%) cases turned out positive. Maximum number of cases belonged to DEN-2 genotype.

Conclusion: Early diagnosis helps in improved patient care, suitable treatment, prevents severe complications and helps limit the spread of the disease. RT PCR is one of the most reliable tests for diagnosis of acute dengue fever.

Key Words: Denv: Dengue virus, DSS: Dengue Shock Syndrome, DHF: Dengue Haemorrhagic Fever,

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VIROLOGY POSTER PRESENTATION ABSTRACTS

P-Viro-0001

DESCRIPTIVE ANALYSIS OF MEASLES CASES PRESENTED TO COMMUNITY PAEDIATRIC OUTDOOR CLINIC, ISLAMABAD, PAKISTAN APRIL-JUNE 2018

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ABSTRACT

Objective: To evaluate the risk factors, clinical presentation, vaccination status of cases and their outcome.

Study Design: A retrospective cross-sectional study was carried out, consisting of children presenting with measles at community paediatric outdoor clinic at Chatha Bakhtawar, Islamabad during April to June 2018. Data containing demographic information, immunization status, clinical presentation, complications and outcome were collected.

Materials and Method: Microsoft Excel was used for data entry while descriptive and inferential analysis of data which were grouped, tabulated and graphed were done using SPSS version 22.0. Variables were tested for association using the chi-square test with level of significance at $p<0.05$.

Results: From a total of 28 cases of Measles evaluated, 14 (50%) were males, with an age range of 5 to 35 months and median age 14 months. Results show that 39% cases were vaccinated for measles while 61% were not vaccinated. Most of the cases (29%) were below 9 months. Common symptoms were fever, oral ulcers and loose stool while pneumonia and gastro-enteritis were common complications. All patients (n=28) were given antibiotics, antivirals and vitamin-A supplementation for treatment. 93% cases recovered and 7% died of complications due to Measles. The analysis indicates a protective association between vaccination and disease outcome and complication.

Conclusion: Measles incidence is high among both vaccinated and unvaccinated children, although fully vaccinated children are relatively protected. Mother's antibodies provide protection for the first 3 months while the first dose is administered at 9 months of age leaving children vulnerable to the disease for some months. Proper monitoring and surveillance of vaccine supply chain resulting in enhanced vaccination coverage are recommended.

Key Words: Measles, Children, Complications, Pakistan

P-Viro-0002

ASSOCIATION OF ELISA BASED SEROLOGICAL MARKERS NS1 ANTIGEN, IGM, IGG ANTIBODIES AND RT-PCR IN THE DIAGNOSIS OF DENGUE VIRUS INFECTION IN PATIENTS ATTENDING DISTRICT HEADQUARTER HOSPITAL RAWALPINDI

ABSTRACT

Objective: To determine the association of **ELISA** based serological markers NS1 antigen, IgM, IgG antibodies and RT-PCR in the diagnosis of dengue virus infection

Material and Methods: In this descriptive study 420 serum samples from patients with suspicion of dengue fever were tested for detection of dengue by NS1 antigen ELISA, IgG, IgM ELISA and RT-PCR for dengue was carried out in all NS1 antigen ELISA positive cases for confirmation of dengue.

Platelet count was recorded in all 420 suspected cases.

Results: Results obtained were analyzed, 249 cases out of 420 samples were positive for either one of the three markers NS1, IgM, IgG: Male to Female ratio was 301 (71.66%): 119(28.33%) respectively. 202 (48.09%) samples were positive for NS1 only, 13 (3.09%) were positive for NS1 and IgG. 07 (1.66%) were NS1, IgM and IgG positive,16 (3.80%) were positive for IgG only ,11 (2.61%) were positive for NS1 and IgM whereas 171 (40.17%) samples were reported negative for NS1, IgM and IgG. RT-PCR was conducted on 233 NS1 positive cases out of which 188(80.06%) cases turned out positive. Maximum number of cases belonged to DEN-2 genotype.

Conclusion: Early diagnosis helps in improved patient care, suitable treatment, prevents severe complications and helps limit the spread of the disease. RT PCR is one of the most reliable tests for diagnosis of acute dengue fever.

Key Words: Dengue virus, Dengue Shock Syndrome, Dengue Haemorrhagic Fever

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P-Viro-0003

A CASE OF MULTIPLE MYELOMA WITH TRIPLE HERPES VIRUS INFECTIONS

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ABSTRACT

Introduction: Multiple myeloma, a rare B cell malignancy is characterized by the presence of monoclonal proteins in serum and/ or urine, lytic bone lesions and decreased serum immunoglobulin levels. Both humoral and cell mediated immunity are compromised in patients receiving treatment for multiple myeloma. Multiple myeloma is generally a disease of elderly and therapeutic strategies include high dose chemotherapy with Bortezomib and the immunomodulatory agents thalidomide and Lenalidomide,

followed by Haematopoietic stem cell transplant (HSCT). Old age, anti-neoplastic drugs and disease related complications increase the susceptibility to infections. Here, we report a case of 62 years old male with multiple myeloma who developed infection with three viruses from herpes family during 1st cycle of chemotherapy.

Case Report: A 62 years old man presented with 10 months history of generalized skin itching, myalgias, weakness and bone pains. He consulted many physicians thereafter but complaints persisted and his condition continued to deteriorate. Eventually, he was thoroughly investigated and was referred to Armed Forces Bone Marrow Transplant Center (AFBMTC) for workup of hemolytic anemia, where he underwent bone marrow aspiration along with trephine biopsy in August 2017. He was finally diagnosed as a case of Multiple Myeloma. He was admitted in AFBMTC and was started on triple regimen which consisted of Bortezomib, Lenalidomide and Dexomethasone. On 11th day of cycle, he developed generalized erythematous rash (Fig 1) followed by fever, bilateral pleural effusion and oral leukoplakia on lateral side of tongue (Fig 2). Multiple investigations were done but all came out to be negative. An oral swab sample was collected from the site of lesion in the mouth and was sent in viral transport medium (VTM) to Virology Department of Armed Forces Institute of Pathology, Rawalpindi. The PCR testing was performed for three viruses belonging to Herpesviridae family; EBV, CMV and HSV-1, due to their strong association with Multiple Myeloma and with the triple regime given for treatment of this disease. The sample was found to be positive for all of the three herpesviruses, simultaneously. Anti-virals started which improved the condition of the patient but attendants took patient to home where his condition deteriorated due to improper care facilities. He expired on 5th day of discharge from hospital**.** The cause of death could not be ascertained due to lack of follow up and was only narrated on telephone by the patient's relatives.

Conclusion: A high degree of suspicion must always be kept in mind while treating patients of Multiple Myeloma on triple regimen; Lenalidomide, high dose Dexamethasone and Bortezomib. Timely testing for viral infections with proper treatment is necessary in such cases to avoid fatal complications.

P-Viro-0004

EMERGENCE OF CHIKUNGUNYA VIRUS IN PAKISTAN: MOLECULAR EPIDEMIOLOGY AND POTENTIAL IMPACT

Nazish Badar, Muhammad Salman, Uzma Bashir Aamir Jamil Ansari. Aamir Ikram

ABSTRACT

Introduction: Chikungunya is a tropical vector-borne disease caused by an RNA virus that has been emerging with significant public health impact, in the last few years. The virus is transmitted by female Aedes mosquitoes (Aegypti and Albopictus). The disease has been associated with a high morbidity including high fever, headache, rash, nausea, vomiting, myalgia and incapacitating arthralgia that gives the disease its name. The objectives of the study were to monitor the clinical features, molecular epidemiology and evolutionary linkages of Chikungunya viruses in Pakistan.

Material and Methods: The descriptive study was conducted from December 2016 to October 2017. Blood samples along with epidemiological information collected from various localities across Pakistan according to World Health Organization's prescribed case definition were tested using CDC Trioplex Real-Time PCR (Dengue, Chikungunya and Zika virus) assay. Sequencing and phylogenetic analysis of chikungunya viruses (CHIKV) viral envelope (E1) and nonstructural (nsp1) protein was carried out and compared with representative sequences from GenBank. Demographic and epidemiological data was gathered and analyzed using statistical software SPSS version 20.

Results: A total of 1448 samples were received at NIH, out of which 50.6% (n=733) were found positive for chikungunya virus by real-time PCR assay. The outbreak started in December, 2016 and reaching its peak during May 2017 with over 37% (n=153/411) cases positive for chikungunya PCR. The mean age of the positive cases were 31.8 +/- 15.7years. The highest incidence rate of the disease was in the patients' aged from 31 to 40 year. The classical presentation includes fever, arthralgia, polyarthritis, muscle pain and skin rashes. Epidemiological and phylogenetic analyses indicated local transmission of the ECSA (East/Central/South African) CHIKV genotype in Pakistan. All analyzed Pakistan CHIKV strains were highly similar with strains from the Indian Ocean lineage within ECSA genotype. ECSA genotype specific substitutions were also found in E1 (T98A, A145T) and Nsp1 (T128K) gene, whereas substitution T98A is also associated with mosquito adaptation determinants in related alphaviruses.

Conclusion: This study has revealed a high incidence of CHIKV particularly in the southern parts of Pakistan and the principal lineage detected during the study was Indian Ocean Lineage within ECSA genotype. Molecular surveillance of chikungunya virus is important as it provides data on the circulating genotypes in the country and enabling activation of preparedness measures for future outbreaks. It is imperative to continue monitoring the extent of transmission and the identification of signature mutations that may facilitate the viral transmission by the mosquito vectors.

P-Viro-0005

INFLUENZA VIRUS SUBTYPING DURING WINTER 2017-2018 BY MULTIPLEX PCR

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ABSTRACT

Introduction: Influenza viruses are rapidly evolving pathogens that cause respiratory tract infections with

significant morbidity and mortality in humans. According to WHO, among 43 million cases of acute respiratory tract illness each year, 4-12% are caused by influenza viruses. Influenza is highly contagious spreading rapidly via aerosols, droplets and direct physical contact.

Objective: To determine the frequency of infection caused by Influenza viruses i.e. Influenza A(H1N1) pdm09, Influenza A(H3N2) and Influenza B in patients presenting with respiratory tract infections i.e. ILI (influenza like illness) and SARI (severe acute respiratory illness). Materials and Methods: 624 samples from patients with respiratory tract infections (both ILI and SARI) were included in the study. Specimens collected from the patients included nasal swabs and throat swabs, which were transported in viral transport medium (VTM) to Virology department, AFIP. Multiplex PCR was done for Influenza A(H1N1) pdm09, Influenza A(H3N2) and Influenza B, using WHO recommended Influenza Real-Time PCR panel.

Results: Multiplex PCR was performed on 624 samples from patients with ILI and SARI. 200 (32%) samples were found to be positive for Influenza viruses. Highest number of positive cases occurred in month of January i.e. 148 (74%) cases were positive in January out of total 200 positive cases.

Conclusion: Influenza viruses are an important cause of respiratory tract illness. The dominant subtype in our set up, during winter 2017-2018, was Influenza A(H1N1) pdm09. Highest numbers of positive cases were recorded in the month of January. People with ILI and SARI should be tested for Influenza virus to avoid unnecessary use of antibiotics.

P-Viro-0006

PREVALENCE OF DENGUE INFECTION IN PATIENTS WITH ACUTE FEBRILE ILLNESS

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ABSTRACT

Introduction: Dengue virus infection is an acute febrile illness endemic in several countries including Pakistan. According to WHO almost 128 countries are known to dengue outbreak. Pakistan is main hotspot for dengue. Four serotypes are present in Pakistan and circulating whole year with peak outbreak between (September-November). Early diagnosis, management is crucial for timely intervention to reduce the morbidity and mortality. The diagnosis of dengue virus infection mainly depends on serological testing and virus isolation.

Objective: The aim of this study is to determine the prevalence of dengue infection with acute febrile illness in population of Sindh Pakistan

Study Design: Cross sectional study.

Place and Duration of Study: It was carried out at Diagnostic & Research Laboratory LUMHS Hyderabad. From August 2017 to July 2018.

Material and Methods: A total of 3490 patients were enrolled in the study from which 2802 was tested for dengue antibodies and 688 for dengue rapid antigen. Data was obtained from the patients presented with acute febrile illness and suspected patients for Dengue viral infection who were requested from the physicians. Dengue IgG/IgM is a rapid immunoassay for the qualitative detection of IgG and IgM antibodies to dengue infection. Dengue NSI Ag can identify dengue virus NSI antigen in serum. Dengue antigen or antibodies were checked by chromatography method.

Results: A total of 3490 patients from which 2802 was tested for dengue antibodies and 688 for dengue rapid antigen. Total 3490 cases 421(12%) was positive for dengue infection. Out of 2802 cases 323(11.52%) was positive for antibodies from which 215(66%) was positive for IgG and 108(34%) for IgM. Out of 688 cases 98(14%) was positive for antigen rapid test and 590(86%) was negative for antigen test.

Conclusion: Accurate diagnosis for dengue infection would contribute to better patient care. Several factors such as unawareness of people, climatic change, and poor surveillance are the major causes of dengue outbreak. Awareness should be delivered through electronic media and Workshops and guiding people for cleanness, uses mosquito repellents, water container is free of mosquito propagation. We will recommend repeating this study at a larger scale and different settings to confirm our findings.

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P-Viro-0007

SEROPREVALENCE OF HIV POSITIVE INDIVIDUALS AT AFIP, RAWALPINDI: AN INSTITUTION-BASED STUDY

Rabia Sajjad, Saifullah Khan Niazi, Misbah Noor, Eijaz Ghani, Ali Rathore
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ABSTRACT

Introduction: The global HIV pandemic has touched all parts of the world. According to WHO, an estimated 36.9 million people were living with HIV at the end of 2017. The incidence, though on the falling trend in some areas of the world, is on the rise in our part of the globe. WHO estimates that in 2016, around 150,000 people were living with HIV in Pakistan. The estimated adult HIV seroprevalence in Pakistan is 0.1%, but in some high risk groups the seroprevalence is more than 5%. Armed Forces Institute of Pathology (AFIP), Rawalpindi, is a reference centre which regularly receives samples from individuals (both Armed forces and Civilians) for HIV testing, in large numbers.

Objective: To determine HIV seroprevalence for past 4 years (from 2014 to 2017) at AFIP Rawalpindi, and to see the gender, age and socioeconomic distribution among the HIV positive individuals.

Material and Methods: This was an institute-based retrospective study. Data of all the cases tested for anti-HIV Antibodies was collected from the database of the institute, Laboratory Information Management System (LIMS), for past 4 years; from 2014 to 2017. All samples from these patients had been tested using a fourth generation, HIV-1/2 ELISA test, which also incorporates testing for p24 antigen in addition to the HIV antibodies. All positive results were tested again by chemiluminescence method on Abbott Architect, for confirmation. All individuals found positive for anti-HIV Antibodies, irrespective of gender and age, were included in the study.

Results: Among positive individuals, 192 (91%) belonged to low income group, 5.3% belonged to middle income group while 3.8% were from high income group. Seroprevalence of HIV is seen increasing over time.

Conclusion: HIV is on the rise in our setup, with increasing trends over past four years. The most frequent victims are males in economically productive age group and most of the positive population is from the low-income group. Mass scale awareness campaigns and other appropriate steps must be planned and executed in time, to minimize this upcoming epidemic.

P-Viro-0008

SEROPREVALENCE OF HIV POSITIVE INDIVIDUALS AT AFIP, RAWALPINDI: AN INSTITUTION-BASED STUDY

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NUCLEAR MEDICINE CENTRE SCIENTIFIC SESSION ABSTRACTS

SS-NMC-0001

ROLE OF RECOMBINANT TSH IN MANAGEMENT OF PATIENTS WITH DIFFERENTIATED THYROID CANCER

Dr Shazia Fatima

ABSTRACT

Recombinant TSH is effective in providing exogenous TSH stimulation for patients with differentiated thyroid cancer on thyroid hormone-suppressive therapy. It allows for detection of thyroid remnant and metastases by radioiodine scan and by serum thyroglobulin determination. The sensitivity and image quality of the WBS are similar after rTSH and after TSH withdrawal in the majority of patients. The equivalent 100% sensitivity of rTSH- and withdrawal-stimulated serum thyroglobulin measurement alone in identifying patients with radioiodine uptake outside the thyroid bed may eventually lead to more extensive use of serum thyroglobulin testing after rTSH, with more selective application of radioiodine whole body scan.

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SS-NMC-0002

TC-99M UBIQUICIDIN(UBI) 29-41---A NEW INFECTION IMAGING AGENT

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ABSTRACT

Infections remain a key cause of morbidity and mortality despite of our better and advanced understanding of microorganisms and availability of antimicrobial therapies. Diagnosis of infection, it's localizing and most important, distinguishing infection from inflammation is crucial for a favorable outcome is a challenging problem. Nuclear medicine imaging, because of its sensitivity, offers an attractive option for diagnosis of focal infections. Over the last so many years, there has been a quest to find an infection imaging radiopharmaceutical which can fulfill the criteria of an ideal infection imaging agent. Various agents and techniques including have been evaluated. These radiopharmaceuticals have varying accuracies in differentiating sterile inflammation from infection. ^{99m}Tc UBi(29-41) is a new addition in the armamentarium of infection imaging radiopharmaceuticals. Ubiquicidin UBI (29-41) is a synthetic antimicrobial peptide fragment reported to be highly infection-specific. The basic mechanism of its antimicrobial activity is interaction between cationic domains of peptide and negatively charged surface of bacteria. The safety of UBI (29-41) labelled with 99mTc has been tested in animals and no adverse effects or toxicity was observed. In humans, it has been found to be highly sensitive and specific agent for localizing infective foci in bone and soft tissues and seems to be a promising agent for the specific detection of infections in humans. However, further studies on larger number of patients and different pathologies are still required before proclaiming 99mTc-UBI 29-41 as a new gold standard an ideal radionuclide for the diagnosis of infection.

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SS-NMC-0003

RADIONUCLIDE THERAPY OF INFLAMMATORY JOINT DISEASES

Dr Akif Ullah Khan

SS-NMC-0004

RADIONUCLIDE THERAPY IN CA. PROSTATE AND NEUROENDOCRINE TUMORS

Muhammad Adnan Saeed

NUCLEAR MEDICINE CENTRE FREE PAPER ABSTRACT

FP-NMC-0001

ACCURACY OF 99M TC LABELLED UBIQUICIDIN (29-41) SPECT/CT FOR DIAGNOSIS OF OSTEOMYELITIS IN DIABETIC FOOT

Muhammad Atif, Fida Hussain
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ABSTRACT

Objective: To determine diagnostic accuracy of ^{99m}Tc labeled Ubiquicidin (29-41) SPECT/CT for detection of osteomyelitis in diabetic foot patients by taking bone biopsy as gold standard.

Study Design: Cross-sectional validation study.

Place and Duration of Study: Nuclear Medical Centre, AFIP, Rawalpindi (Six months)

Material and Methods: Study assessed 122 patients of both genders, aged between 30-80 years (mean age = 55.3years), presenting with diabetic foot ulcers having suspicion of osteomyelitis, by ^{99m}Tc-Ubiquicidin (29-41) SPECT/CT followed by bone biopsy and histopathology examination taken as gold standard.

Results: Among 122 patients [94 male (77%) and 28 female (23%)], osteomyelitis was histopathologically confirmed in 113 patients. 107 patients were positive for osteomyelitis on ^{99m}Tc-UBI (29-41) SPECT/CT (True Positives) while false negative were 6 with one false positive. True negative cases for osteomyelitis were 8. Out of which 4 had soft tissue infection without osteomyelitis. The ^{99m}Tc-UBI (29-41) scan showed 94.6% sensitivity, 88.89% specificity, 99% positive predictive value, 57% negative predictive value with overall 94.2% diagnostic accuracy.

Conclusion: ^{99m}Tc labelled Ubiquicidin (29-41) SPECT/CT scan can precisely localize infective focus, in diabetic foot osteomyelitis, with simultaneous discrimination between bone and soft tissues. This diagnostic performance was unaffected by patient's age and gender.

Key Words: Diabetic foot, Osteomyelitis, ^{99m}Tc-UBI (29-41) SPECT/CT, Diagnostic Accuracy

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FP-NMC-0002

DIAGNOSTIC ACCURACY OF 99M TC METHOXYISOBUTYLISONITRILE (MIBI) SCINTIMAMMOGRAPHY IN DETECTION OF BREAST CANCER

Rabeeca Sharoon, Fida Hussain, Saima Seher, Inam Farsi, Ali Jamal
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ABSTRACT

Objective: To evaluate the diagnostic accuracy of Scintimammography as a reliable diagnostic modality for detecting carcinoma breast keeping histopathology as a gold standard for final diagnosis.

Study design: Cross sectional validation study.

Setting and Duration of Study: Nuclear Medical Centre (NMC) Armed Forces Institute of Pathology (AFIP), Rawalpindi. Study was carried out from Oct 2016 till April 2017 for a period of 06 months.

Materials and Methods: 738-1100 MBq (20-30 mCi) of ^{99m}Tc MIBI was injected intravenously in the contralateral arm to the affected breast. In case of bilateral breast lumps tracer was injected in foot. Scintimammography was performed at 20 min and 60 min. Planar Images of 10 minutes duration were acquired in prone and supine positions.

Gamma camera was equipped with low energy all-purpose collimator. Photopeak centered at 140 Kev (Kiloelectron volt) with 20% window, and matrix size of 256X256 pixels.

Results: A total of 99 patients fulfilling the inclusion criteria underwent Scintimammography out of which 2 patients had indeterminate results and 1 had a positive result due to metastasis from Ca ovary in breast these fulfilled the exclusion criteria, thus a total of 96 patients were included in the study. After scintimammography each patient underwent FNAC/ TRUCUT for histopathological diagnosis. The age of patients ranged from 20-74 years. The mean age of presentation was 43.22 years. Diagnostic accuracy was calculated for 96 patients. 53 had true positive (TP) while 35 had true negative (TN) results. The sensitivity, specificity, **PPV** and **NPV** of the study was 98.1, 88.1, 91.4, 97.4% respectively and an accuracy of 93.75%.

Conclusion: Scintimammography is an additional tool for diagnosis of Ca breast with a diagnostic accuracy of 94%.

Key Words: Scintimammography, Breast Cancer, ^{99m}Tc MIBI, Breast Imaging

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FP-NMC-0003

I-131 MIBG MYOCARDIAL SCINTIGRAPHY FOR DIFFERENTIATION BETWEEN PD AND MSA

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ABSTRACT

Objective: The aim of this study was to differentiate between Parkinson's disease (PD) and multiple system atrophy (MSA) by utilizing I-131 MIBG myocardial scintigraphy.

Material & Method: Patients with neurological disorders like PD and MSA were referred to this center to differentiate between PD and MSA. Myocardial scintigraphy with I-131 MIBG was performed and heart/mediastinum (H/M) uptake ratios were calculated for each at 15 minutes and 4 hours after injection. The washout ratio (WOR) of

MIBG was also calculated. H/M ratio of greater than 1.80 and WOR of less than 15% were taken as cut off values.

Results: The results showed that 12 patients had low uptake and high washout ratio and were labelled as PD. Rest of the 8 patients had uptake within the normal range and were labeled MSA. The diagnosis was later on confirmed through follow up of the patients with neurologists.

Conclusions: Myocardial scintigraphy with I-131 MIBG can help distinguish patients with PD from those with MSA with good sensitivity and specificity enabling early differentiation between PD and other Parkinsonian disorders.

Key Words: ^{99m} Tc MIBI, myocardial scintigraphy

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