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Oral presentations

Relation of thyroid cancer and anthropometric factors in Pakistan

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Introduction and objective: We have investigated the relationship of thyroid cancer (TC) and anthropometric factors in Pakistani population as this association remains to be controversial.

Methods: A total of 172 TC patients admitted at Aga Khan University Hospital (AKUH) from 2000 to 2014 were included. Patient's demographics, tumor subtypes, body weight, height, body mass index (BMI), body surface area (BSA) and body fat percentage (BF%) were calculated.

Results and discussion: Out of 221 patients, 172 patients were included in this study. Mean age was 43 years \pm 15.40. Among all subtypes of TC, papillary remains the most common (72.1%), followed by medullary (10.5%), follicular (7.6%) and others (9.9%). Excess BMI > 23 kg/m², BF%, BSA, weight and height were significantly associated with TC incidence in overall population (p-value < 0.01*). In women, BF% was found to be in obese range (mean = 35.07% \pm 7.6, p-value \leq 0.01*). In men, height > 160 cm and weight > 60 kg were positively associated with TC. No significant relation was seen between gender and BMI.

Conclusions: Excess BMI > 23 kg/m² and age > 40 years were positively associated with TC. In women, increased BF% and in men, height > 160 cm and weight > 60 kg had a significant

relation with TC incidence.

Expression alterations of metabolic proteins in schizophrenic brain regions

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Introduction: Schizophrenia (SZ), a multifactorial disorder exhibits diverse neuropathological aberrations including defective neurotransmission system, neuroanatomical abnormalities and impaired synaptic connectivity. Along with these complications there is surfacing evidence that metabolic dysfunction and energy deficit may also contribute to its pathophysiology.

Methods: Present study entails, differential proteomic analysis of autopsied schizophrenic brain substantia nigra, cortex, and hippocampus (n=7) by using two dimensional electrophoresis (2DE) followed by Orbitrap mass spectrometry identification. Differential expression was validated by western blot, while In Silico analysis was applied for functional interactive role in metabolism.

Results: Proteome mapping of SZ patients revealed more profound alterations in SN protein expression than other brain regions compared to controls. SN exhibits differential expression of six proteins, enriched in several energy metabolic pathways including decreased expression of phosphoglycerate mutase 1, ATP synthase subunit d, mitochondrial and malate dehydrogenase cytoplasmic, while increased expression of glyceraldehyde-3-phosphate dehydrogenase, 4-trimethylaminobutyraldehyde dehydrogenase, and alcohol dehydrogenase were found as compare to normal brain. Cortex and hippocampus also portray significant variations in expression patterns of five proteins across brain of schizophrenia patients.

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¹ Please note that the selected abstracts were chosen by the editors and this publication does not include all abstracts presented at the congress.

Conclusions: Identification of differentially expressed metabolic proteins provides an insight for a better understanding of schizophrenia pathophysiology. Further characterization, will elucidate the functional mechanisms underlying defective metabolic pathways, which will provide promise for future therapeutic developments in the treatment of schizophrenia.

Analyses of pre-mRNA splicing machinery by UV-induced protein-RNA cross-linking and mass spectrometry

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Introduction and objectives: The human genes that encode proteins undergo pre-mRNA splicing, an essential step of gene expression in which noncoding sequences are removed and coding sequences are ligated together. Unraveling splicing mechanism at the molecular level is not only important for understanding gene expression, but it is also of medical relevance, as aberrant pre-mRNA splicing is the basis of many human diseases or contributes to their severity. Although methods based on high-throughput sequencing have advanced our ability to identify the specific RNAs bound by a particular protein, there is a need for precise and systematic ways to identify RNA interaction sites on proteins.

Methods: We have developed an experimental workflow combining UV-induced cross-linking and high-resolution mass spectrometry for the identification of protein-RNA cross-linking sites. Purified complexes were UV irradiated at 254 nm and proteins therein hydrolyzed with trypsin. Peptide-RNA cross-links were enriched by TiO₂ chromatography and analyzed by ESI-MS. Data analysis for identification of putative cross-links was performed by novel RNPxl pipeline.

Results: The workflow not only enables the systematic identification of cross-linked peptides but also amino-acid residues in RNA-interacting domains of proteins.

Conclusions: Successful identification of several cross-linking sites from human and yeast splicing complexes make it a promising technique to study any RNA-protein complex of interest.

Crosstalk between cytoskeletal and metabolic proteins: A roadmap to ad pathology

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Introduction and objectives: Cellular activities are based on complex networks of protein interactions. Identification and interaction analysis of associated protein partners is the basis of many biological studies, determining the key etiological factors. Alzheimer's disease, a pathologically complex and multifactorial disease is the most common cause of dementia. It is characterized by a progressive decline in memory and cognitive functions. Aberration of multiple pathways is observed in Alzheimer's disease including metabolic pathways which subsequently alter the allied cytoskeletal system. This study is designed to unveil the cytoskeleton and metabolic proteins interaction in AD and interpret their association.

Methods: Human autopsied brain tissue samples from normal age matched control (n=6) and AD patients (n=5) were procured. Two dimensional Blue Native/SDS PAGE combined with Orbitrap mass spectrometry analysis was accomplished to determine the interacting cytoskeleton and metabolic proteins. The interaction was further confirmed by co-immunoprecipitation.

Results: A total of thirteen protein complexes were obtained on BN-PAGE which resolved into many crucial proteins of different

cellular networks. An important observation was a complex comprised of novel interacting partners including glyceral-dehyde-3-PO4 dehydrogenase, actin cytoplasmic, microtubule associated protein 1B and glial fibrillary acidic protein. Further the interaction between actin and GAPDH confirmed by co-immunoprecipitation has not been established before in AD.

Conclusion: In accordance with the previous findings of GAPDH and actin differential expression and post translational modification, our results suggest interesting implications of these two proteins in AD.

Detection of high risk human papilloma virus (HPV) genotypes 16/18 in oral lesions of tobacco chewers in Pakistan

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Introduction and objective: The Human Papilloma Virus (HPV) has evolved as a new culprit of malignant and premalignant oral lesions. The objective of this study was to find out the frequency of HPV and its high risk genotypes in different lesions of oral cavity of tobacco chewers.

Methods: From 492 subjects (421 males and 71 females), 20 ml of oral rinse sample was collected after obtaining an informed consent. Normal subjects with no chewing habits (250) including 135 males and 115 females were also taken from same setting. Gentle brushings over the lesions with the help of dental floss brush and stored at 4 °C until DNA extraction. DNA was extracted and PCR was performed using HPV consensus primers Gp5+/Gp6+ and HPV 16, 18 specific primers for genotyping. Categorical data was calculated as frequencies and percentages.

Results: Oral pre-malignant lesions were present in 421 (86%) males and 71 (14%) females having leukoplakia (173, 35%), erythroplakia (60, 12%), submucous fibrosis (192, 39%) and L/E (67, 14%). Total number of HPV positives were 128 (26%), having HPV 16 (13%) and HPV 18 (11%) whereas, 76% had other genotypes. Among submucous fibrosis 82 (46%) were HPV positive. Out of total 128 HPV patients 92% were males and 8% were females. All controls were found to be HPV negative.

Conclusion: Frequency of HPV was found high (26%) in oral lesions with HPV16/18 as 13% and 11% respectively. The patients with submucous fibrosis are at greater risk of having HPV. Other HPV genotypes causing premalignant lesions require further investigation.

Diagnostic, prognostic and predictive value of microRNA-21 in breast cancer patients, their daughters and healthy individuals

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Introduction: MicroRNA-21 (miR-21) located on 17q23.1 has shown high expression in breast cancer. This gene has anti-apoptotic ability and causes tumor cell growth. It is also normally involved in functions such as signal transduction pathways affecting normal cell growth and differentiation.

Objective: To determine the presence of miR-21 in the serum levels of breast cancer patients, their daughters and healthy individuals.

Methods: A total of 132 subjects were recruited: 50 breast cancer (cases), 50 age-matched healthy individuals (control A) and 32 daughters of index cases (control B). The sampling technique used for cases was random sampling. Controls were selected by purposive method. Serum tests were run on qRT PCR and threshold cycle was determined and fold change calculated.

Results: Fisher exact test was used when analyzing between cancer patients and healthy patients or with their daughters. Normality of continuous variables was assessed by Shapiro Wilk's test. While comparing the same between two groups, student t-

test and Mann-Whitney test was used. P-value ≤ 0.05 was considered significant. MiR-21 was significantly higher in cases as compared to control A and B (p-value=0.001). However control B showed significant gene expression as compared to control A (p-value=0.001). The 2ct formula was applied to compare the fold change between the groups. The cases were also divided into ER, PR and HER2 positive and negative cases. It was observed that triple negative cases showed a greater expression of gene as compared to other groups (p-value=0.001).

Conclusion: High expression of miR-21 in breast cancer patients suffering from stage III invasive ductal carcinoma had been calculated as compared to its age matched healthy subjects. This result can mark miR-21 as a potentially strong diagnostic and prognostic biomarker of breast cancer. MiR-21 profile was also studied in daughters of the index cases. Their expression was also shown to be significantly higher as compared to the healthy individuals but lesser than the full blown disease of breast cancer. This result strengthens the concept of inheritability of this disease and this gene can also be labeled as a predictive biomarker. When Ct-values and fold change calculations were compared between ER, PR and HER2, higher expression was seen in negative tumor tissues which are very resistant tumors and respond poorly to chemotherapy.

Serum metabolomic profiles for breast cancer diagnosis, grading and staging by gas chromatography-mass spectrometry

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Introduction and objectives: Detection of metabolic signature for breast cancer (BC) has the potential to improve patient prognosis. This study identified potentially significant metabolites differentiating between breast cancer patients and healthy controls to help in diagnosis, grading, staging and determination of neoadjuvant status.

Methods: Serum was collected from 152 pre-operative breast cancer (BC) patients and 155 healthy controls in this case-control study. Gas chromatography-mass spectrometry (GC-MS) was used to obtain metabolic profiles followed by chemometric analysis.

Results: Chemometric analysis showed the identification of significantly differentiated metabolites including 7 for diagnosis, 19 for grading, 23 for staging, 15 for stage III subcategories and 10 for neoadjuvant status (p value < 0.05). Partial Least Square Discriminant Analysis model revealed a distinct separation between healthy controls and BC patients with a sensitivity of 80% and specificity of 100% on external validation. Models for grading, staging and neoadjuvant status were built with Decision Tree Algorithm with predictive accuracy of 71.5%, 71.3% and 79.8% respectively.

Conclusion: Pathway analysis revealed increased glycolysis, lipogenesis, and production of volatile organic metabolites indicating the metabolic alterations in breast cancer.

Growth factors mediated preconditioning of mesenchymal stem cells: Strategy for enhancing renal tissue regeneration

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Introduction and objectives: Acute kidney injury (AKI) is a complex disorder manifested by rapid loss of renal function. AKI has strong influence on expression of renal specific genes which may play central roles in key cellular pathways and targeting these pathways may turn out to be effective therapeutic option. Transplantation of mesenchymal stem cells (MSCs) has been shown to improve kidney function following injury. However, poor survival and grafting of stem cells to the site of injury has restricted their therapeutic efficacy. Preconditioning of stem cells can be a potential strategy; chemokines and growth factors can improve their potential in terms of homing and differentiation. Objectives of the present study were to examine the changes in the expression profile of genes associated with renal injury and to explore the novel strategy of preconditioning of MSCs for injured kidney cells.

Methods: Rat AKI model was established with gentamicin and renal specific genes were analyzed through RT-PCR. For preconditioning strategy we used IL-7 gene to transfect MSCs. MSCs were co-cultured with cisplatin-treated injured Mardin-Darby bovine kidney (MDBK) cells and their in vitro fusion potential was analyzed.

Results: Several genetic changes occur in AKI that may contribute in long term renal injury consequences and targeting these pathways may appear to be effective therapeutic options. Flow-cytometry of preconditioned MSCs and injured MDBK cells revealed significant (P ≤ 0.001) cell fusion compared to that of the normal MSCs. In addition, we also observed improved migration ability of preconditioned MSCs in the in vitro wound healing assay.

Conclusions: For efficient stem cell therapy, it would be valuable to determine the contribution of various cellular factors in the fusion process so that a rationale for the use of such preconditioned cells can be determined.

Amino acid racemization in human dentine as an indicator of chronological age – A study in Karachi, Pakistan

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Introduction and objectives: Amino acid racemisation is a reliable method to estimate age in developed countries. This study was designed to determine the coefficient of amino acid racemisation (AAR) with reference to age in our population. The objectives are to determine the coefficient of aspartic acid racemisation (AAR) with reference to age by HPLC, to measure the mean error in calculating age using this method; and to identify any differences from earlier observation and discuss the reproducibility in our setting.

Methods: Hundred (100) teeth were obtained from subjects between the age of 11 and 70 years who had come for routine extraction procedure or for orthodontic procedures. Incisors, canines, premolars and, first and second molars were included. Carious tooth and third molar were excluded. Samples were stored, dried, de-mineralized, hydrolyzed, and derivatised. High-Pressure Liquid Chromatography (HPLC) was performed to quantify the L- and D-forms of aspartic acid in dentin. Correlation and regression was then tabulated based on the quantification, and was compared with data from other studies.

Results: It was observed that there is very strong positive correlation (0.818) observed between actual age and co-efficient of racemization (statistically significant at 5%), which indicates that as age increase co-efficient of racemization increases. Based on the analysis of 85 samples to estimate age least Square method was used to derived the regression line i.e. Age = $-4.391 + 347.396$ (co-efficient of Racemization). Co-efficient of determination was found to be 0.74, which show that regression equation for estimating age was 74% correct. The computed regression line was tested by using coefficient of racemisation for 15 samples, we observed that the mean of actual age (38.44 ± 13.22) was not significantly different

from the mean of calibrated age (37.52 ± 12.36) which is estimated by regression equation (P -value=0.222). The mean error (difference between actual and calibrated age) is found to be 0.911 ± 7.41 .

Conclusions: The correlation coefficient was strongly positive. The results strongly suggests that aspartic acid racemisation of human dentine is a precise method for estimation of chronological age in living and dead. The methodology should be standardized to make the results more accurate and prevent fallacies. However, it remains an expensive procedure, considering that the laboratory equipment is not available in most cities of Pakistan.

Correlating vitamin d deficiency with volumetric breast density via a fully automated soft ware Volpara™ in the reproductive age group

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Objective: To see the correlation of volumetric breast density and Vitamin D deficiency in the reproductive age group.

Methods: We enrolled 300 patients from the general surgery outpatient department, their blood samples were collected and vitamin D level was determined via chemiluminescence Method. Full field digital mammography was done and the DICOM images were tagged and processed via fully automated software Volpara™ by using the formula "Volumetric breast Density = $100 \times \text{Volume of fibroglandular tissue (cm}^3\text{)} / \text{Volume of breast tissue (cm}^3\text{)}$ ".

Results: There is enough evidence now that Vitamin D deficiency is inversely related to mammographic density and MD itself is a strong predictor of breast cancer risk. Measurement of volumetric breast density objectively via a fully automated software "Volpara" and using the Raw Processed version of the FFDM in this study was attempted for the first time in Pakistan and correlating with the vitamin D deficiency and also BI-RADS (Breast-Imaging reporting and Data system) which is a common method used in clinical practice worldwide. The total number of patients had only two women with normal vitamin D level, the deficient group was evaluated. In the group of women with low Vitamin D (Vitamin D < 50), 76% had elevated VBD and 24% had normal VBD. The test for proportions compares whether these two groups are significantly different in size or not.

Conclusions: The null hypothesis assumes that vitamin D deficient women have the same number of people in tolerable and excess groups. Because the p -value is small, the null hypothesis is rejected and we conclude that the number of women with elevated breast density is not the same as the number of women with normal breast density, it is higher.

Natural compounds suppress oxidative stress and inflammatory response in activated mouse macrophages

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Introduction: Inflammation is quite complex, initiated by several factors includes, molecules ranges from bacteria to chemical causing cellular trauma. Tissue injury induced by this trauma results in the inflammatory mediators release by macrophages, including reactive oxygen species (ROS) like superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), nitric oxide and increased expression of pro-inflammatory cytokines, NF kappa B, and inducible Nitric Oxide Synthase. A current study has led to the isolation of two new Acylphloroglucinols derivatives along with four known compounds, Myricetin, Isousnic acid, G3-factor and myrto-compulone E from Myrthus.

Methods: In this study, we used Lipopolysaccharide induced macrophages to study immune mediators release in culture

supernatant. We also sought to further delineate the underlying mechanisms elicited by oxidative stress and inflammation and suppressed by compounds.

Results: The results show that new Acylphloroglucinols derivatives and Myricetin significantly suppressed the oxidative stress and anti-inflammatory efficacy in suppressing, total reactive species (ROS), superoxide, nitric oxide, pro-inflammatory inducible nitric oxide synthase (iNOS), proliferation of T-cells and nuclear factor-kappa B (NF-κB) nuclear translocation. All the compounds were also non toxic to other cell systems.

Conclusion: Based on these results, we conclude that natural product may contribute in anti-inflammatory drug discovery.

Imipenem resistant pseudomonas aeruginosa: the fall of the final quarterback

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Introduction and objectives: To isolate, determine the frequency, and study the demographic trends of MBL positive Pseudomonas aeruginosa from imipenem resistant isolates collected from clinical samples in a tertiary care hospital of Pakistan.

Methods: In this cross sectional study a total of 230 strains of Pseudomonas were isolated from various clinical specimens on the basis of culture and biochemical tests. Imipenem resistant isolates were selected by Kirby Bauer Diffusion technique, followed by screening for MBL production by Imipenem EDTA Combined Disc Test. Demographic details of each patient were recorded on a separate questionnaire. Chi-Square goodness-of-fit test was computed to review the isolation of MBL positive isolates (P -value ≤ 0.05) in different specimen.

Results: Out of 230 strains of P. aeruginosa 49.5% were imipenem resistant; MBL production was confirmed in 64.9% of the resistant isolates. Resistance to polymyxin B (12.5%) was notable. Majority of the MBL positive strains were isolated from patients aged between 20 and 39 years (45.9%) and the predominant source was pus (43.24%) which was found to be statistically significant (P -value=0.04). Outpatient departments (24.3%) and burn unit (21.6%) were the major places for resistant isolates.

Conclusions: MBL production is one of the major causes of IRPA. Increasing resistance to polymyxin B is grave. Due to acquisition of MBL strains, MDR P. aeruginosa has become endemic in tertiary setups.

Antibiotic resistance in neonatal sepsis – study on low birth cases from Karachi-Pakistan

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Introduction and objectives: Premature neonates are often immunocompromised and readily susceptible to infectious diseases. They are deficient in antibodies whereas neonatal neutrophils are immature and depleted (2 fold in circulatory pool). Neonatal anatomic barriers are susceptible and hence infection control poses significant challenge. The objective of this study is to assess the common pathogens isolated in neonatal sepsis and specify the antibiotic resistance pattern.

Methods: The study was conducted in the neonatal ICU of two public sector tertiary care hospitals inclusive of 234 cases. Premature neonates with clinically confirmed bacterial infection were included comprising of 48.6% cases of Early onset neonatal sepsis (EONS) with obstetrical complication and fulminant, multisystem disease. Intrapertum fever was recorded in 87% cases of EONS among which, 22% culture positive neonates were initially asymptomatic. Apnea, fever and metabolic acidosis were the more

prevalent symptoms. CBC, C-reactive protein, Glucose and Blood culture test were recorded.

Results: The most common pathogen was Group B streptococci (24%), *Klebsiella pneumoniae* (21%) and *E. coli* (15%). Fetal hypoxia was confirmed in 34% cases. Ampicillin and Gentamycin was given empirically in 87% cases. Modified Kirby Baur disc diffusion technique was used to determine antibiotic resistance. *Klebsiella pneumoniae* was greatly resistant against cephalosporins (average of 54%) and co-trimoxazole (87.9%) whereas comparatively susceptible to quinolones (ciprofloxacin). Resistance of amikacin was elucidated in 87% cases.

Conclusion: In lieu with preventive approaches to avoid neonatal sepsis, careful treatment choice of antibiotic is required to deplete chances of therapeutic failure.

Withania coagulans: A potential cholesterol lowering plant

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Introduction and objective: *Withania coagulans* has many medicinal properties. Currently its cholesterol lowering effect was investigated in terms of HMG-CoA reductase inhibiting activity.

Methods: The study was designed to investigate in-vivo effect of methanolic fruits extract (MFEt) of *W. coagulans* in hyperlipidemic rabbits. On the completion of trial, total cholesterol (TC) and HMG-CoA/mevalonate ratio were estimated. Additionally, in-silico study was performed to find out the inhibitory effect of 21 chemical compounds present in fruits of same plant on HMG-CoA reductase (HMGCR). Molecular docking calculations were carried out by using Molegro Virtual Docker (MVD) software.

Result: All doses of MFEt were significantly ($p < 0.05$) decreased TC and improved HMG-CoA/mevalonate ratio indicating HMGCR inhibition. Interestingly, 5 compounds were displayed the highest MolDock score including Ergosta-5, 25-diene-3 β , 24 ϵ -diol, Withacoagulin, Withanolide D, Coagulin D and Withaferin. Of which, excluding Withanolide D rest of the 4 compounds interacted with catalytic residues present in the active site of HMGCR by forming hydrogen bonds and inhibited the activity of same enzyme.

Conclusions: The present in-vivo and in-silico studies proved that compounds present in fruits of *W. coagulans* are predicted as potent inhibitors of HMGCR that could be used for the development of drug for hypercholesterolemia.

Cardioprotective effect of modified bone marrow mesenchymal stem cells after transplantation in rat model of myocardial infarction: An applied approach towards cellular therapy

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Introduction and objectives: Myocardial infarction is increasing cause of death worldwide. In South Asia, cardiac diseases are increasing at a rate greater than in other part of the world. Regenerative medicine involves the repair or regeneration of an organ, tissue or cells in order to restore an impaired function of the tissue. The cellular therapy offers a promising therapeutic strategy for the treatment of different diseases including myocardial infarction. Mesenchymal stem cells (MSCs) are having immense importance in the emerging field of regenerative medicine and have tremendous capability for cellular therapy and tissue engineering. MSCs can be converted into cardiomyocytes by treating them with DNA demethylating agents. Our aim is to investigate the cardioprotective effect of pretreated MSCs when transplanted in infarcted myocardium of rat.

Methods: In the present study, rat mesenchymal stem cells were treated with DNA-demethylating agents. The optimized

concentrations of these compounds were added separately into the culture medium and the treated cells were analyzed for the expression of cardiac specific genes by RT-PCR and cardiac specific proteins by immunocyto-chemistry and flow cytometry. The treated MSCs were then transplanted to the rat model of myocardial infarction and cardiac functional studies were performed by echocardiography.

Results: We have found expressions of cardiac specific proteins and genes in the modified mesenchymal cells both at genetic and protein levels. Transplantation of modified MSCs into infarcted myocardium of rat improved the cardiac left ventricular function possibly by enhancing the rate of bone marrow stem cell differentiation into mature cardiomyocytes in the infarcted heart.

Conclusions: We therefore, conclude that pre-treating autologous mesenchymal stem cells before transplantation may increase the likelihood of successful regeneration of damaged myocardium. The study put forth another valuable aspect that would serve as a tool for modified cellular therapy.

Antiplatelet aggregating and membrane stabilizing properties of areca nut

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Introduction and objectives: Platelet aggregation inhibitors are one of the key preventive and therapeutic agents for cardiovascular diseases. Areca nut is used in folk medicine for treatment of various ailments. Present study was conducted to investigate the antiplatelet aggregating and membrane stabilizing activities of areca nut extract and its fractions using platelet aggregation and membrane stabilization assays.

Methods: To determine the effect of areca extract and its fractions on platelet aggregation, blood was taken from healthy human volunteers and platelet aggregation was monitored. Membrane stabilizing properties were assessed using human red blood cells.

Results: Our results showed that areca extract was effective against arachidonic acid induced platelet aggregation in dose dependent manner leading to complete inhibition at 10 mg/ml dose. Data obtained from the present study showed that the areca extract (EC₅₀ = 91 μ g/ml) and its aqueous fraction (EC₅₀ = 55 μ g/ml) possess significant membrane stabilizing activity in a dose dependent manner (IC₅₀ = 100 μ g/ml).

Conclusions: These findings suggest that areca nut extract, ethylacetate fraction and aqueous fraction possess significant antiplatelet aggregating and membrane stabilizing properties, and aqueous fraction was most effective which may be of clinical value. However, further studies are required to identify its active compound(s) via bioassay guided-fractionation method.

Small molecular activators of proteasome-related HsIV peptidase

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Introduction and objectives: The HsIVU or prokaryotic mini-proteasome is two component system based on HsIV peptidase and HsIU ATPase. Like proteasome it is involved in intracellular proteins degradation. Due to its occurrence in pathogenic microbes and absence in human beings, it is considered as a potential antimicrobial drug target. The functional HsIVU complex forms when HsIV dodecamer is flanked at both sides by HsIU hexamers and the carboxy termini of HsIU subunits intercalate into a clefts between two adjacent HsIV subunits. This intercalation is followed by conformational change in HsIV active site resulting in its allosteric activation. We for the first time report certain small

molecules which are capable of activating HslV peptidase in the absence of its natural activator, HslU.

The benzimidazole, quinazoline and chromone derivatives were suggested by ligand docking studies to bind at the carboxy terminus binding pocket of HslV in a manner similar to HslU carboxy terminus. This was confirmed by HslV activation assays with these compounds that gave ED50 in micromolar range (0.6–1.5 μ M).

Results: The results showed that small, extracellular non-peptidic molecules can allosterically activate the peptide hydrolytic activity of HslV, which in turn would initiate intracellular proteolysis.

Sensitivity pattern of *Moraxella catarrhalis* isolated from hospitalized patients

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Introduction and objectives: *Moraxella catarrhalis* (formerly known as *Branhamella catarrhalis*) has emerged as a significant bacterial pathogen of humans over the past two decades. Over the same period, studies have revealed its involvement in respiratory (e.g., sinusitis, otitis media, bronchitis, and pneumonia) and ocular infections in children and in laryngitis, bronchitis, and pneumonia in adults. It has discovered an increasing number of B-lactamase-positive strains. We aim to find out the sensitivity pattern of *Moraxella catarrhalis* isolated from sputum.

Methods: Total 124 sputum samples were analyzed. Confirmation done by using chocolate agar a typical golden yellow which can be distinguished by hockey puck like colony. Further identifications were done by gram staining, oxidase test and biochemical test. Antibiogram was done by CLSI method. Statistical analysis was also performed using SPSS version 17.

Results: Sensitivity pattern showed AMC 99%, TE 49%, CRO 01%, E 30%, SXT 04%, CIP 50%, CEF 84%.

Conclusions: To control the resistivity of these organisms some preventive measurements like proper identification and selective medication should be taken.

Assessment of HCV genotypes gene flow hybridization and its comparison with conventional PCR

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Background: Nucleotide sequence analysis of hepatitis C virus (HCV) strains showed substantial variability leading to a classification into several genotypes and subtypes.

Objective: The objectives of the present study were the knowledge of the HCV genotypes in Pakistan, and determining their prevalence.

Methods: During the course of this study, HCV-RNA positive sera samples from 200 chronically infected patients were characterized by genotyping assay and Automated Sequence Analysis. Genotyping assay utilized type-specific primers for amplification of the core region, where as sequencing was done for 5'Noncoding region of HCV using ABI 3100 Sequencing Analyzer.

Results: During our study we came across that genotype 3a was the most prevalent (36%) followed with 3b (26%), 3c (3.5%), 3d (4%), 1a (2.5%), 1b (1%), 1c (2.5%), 1d (0.5%), 2a (12%), 2b (0.5%), 2c (0.5%), 4 (0.5%) and 4a (1.5%). Genotypes 3d, 1c, 1d and 2c were reported for the first time from Pakistan.

Conclusion: Advances in the field of molecular biology have provided rapid diagnostic tools that have reduced the turnaround times for detecting HCV genotype by using "Flow-through" hybridization in Pakistan.

Galectin-3 and Wnt signaling in myocardial infarct healing: Is there a connection?

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Introduction and Objectives: Myocardial infarction (MI) leads to complex repair processes including scarring and fibrosis. Galectin-3 (Gal-3) is a beta-galactoside binding lectin responsible for cardiac fibrogenesis and adverse left ventricular (LV) remodeling. Canonical Wnt signaling in myofibroblasts following MI also modulate wound healing and fibrosis. As Gal-3 and Wnt 3a play a significant role in the post MI sequel we aim to measure their concentration in murine heart at 7 week post Ischemia/reperfusion injury and to determine their correlation.

Methods: Male C57B6/J mice were used for myocardial IR injury by temporarily ligating the left anterior descending artery of the heart for 30 min to create ischemia/infarction followed by restoration of blood flow. Samples collected 1 week post IR injury point was processed for ELISA to detect Gal-3 and Wnt-3a levels in the heart.

Results: Gal-3 and Wnt 3a levels were significantly increased in the LV heart tissue at 1 week post IR compared to sham operated mice. There was a strong and significant positive correlation observed between Gal-3 and Wnt-3a at 1week post IR injury.

Conclusions: Gal-3 and Wnt-3a levels were increased in 1 week post myocardial IR injury in conjunction with intrinsic cardiac repair. Understanding the mechanisms of this repair process could lead to optimal management and prevention or delay in the onset of heart failure.

STD pathogens determined in semen using PCR and "flow-through" hybridization technology

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Background: The prevalence of sexually transmitted Disease (STDs) in health care facilities for diagnosis of common pathogens, those causing infertility like *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma hominis*. Genital wart is a highly contagious sexually transmitted disease caused by some sub-types of human papillomavirus (HPV).

Methods: Semen samples were obtained by masturbation into sterile containers after sexual abstinence of 48–72 h. Samples were subjected to semen analysis within one hour of collection. The concentrations of sperm as well as sperm motility were also determined. DNA extraction was extracted of all the samples and the PCR assay was performed. The amplicons are subsequently hybridized to pathogen-specific capturing probes via "Flow-through" hybridization.

Result: During our study we came across with the STI pathogens present in our population and the reason for infertility was the main cause. When *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were detected in their wife's were also screened and these STI pathogens were identified.

Conclusions: The main route for the transfer of STI pathogens were the men special those who visited commercial sex workers as they were working in other cities and the complained for infertility. Screening for bacterial STI pathogens, *Mycoplasma hominis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are strongly recommended because these pathogens can cause serious reproductive complications.

Effect of Citrus paradisi on coagulation mechanism

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Introduction and Objectives: Over the past few years, herbal remedies and medicinal plant entities are of great interest in various region of the world for their therapeutic and preventive effects in various cardiovascular ailments like atherosclerosis. Numerous animal models were studied extensively for elucidation

of these plant component effects on different organs. Citrus paradisi (*C. paradisi*) due to its antioxidant and anti-inflammatory potential has been selected in this study to evaluate the effect of this compound on coagulation and anticoagulation factors. *C. paradisi* contains high concentrations of hesperidin, naringin, limonene and related flavonoids.

Methods: Doses were selected in the range of 0.1–0.5 ml/kg. Aspirin and warfarin 150 mg/kg and 5 mg/kg–10 mg/kg respectively were used as standard anticoagulants. Animals were divided into six groups with ten rabbits in each group.

Results: At median dose level, significant enhancement in thrombin, pro-thrombin time was observed in comparison to control, while fibrinogen levels were significantly reduced with warfarin. Furthermore, substantial inhibition of platelet aggregation by adenosine phosphate, collagen, epinephrine and arachidonic acid was observed at median *C. paradisi* dose (MCPD).

Conclusions: Results of this study have shown an effective anticoagulant activity. Thus the patients, who are at risk of cardiovascular event development may additionally be advised to consume grape fruit juices in their diet to reduce the chances of mortality and associated morbidities.

Conventional clinical and prognostic variables in 150 oral squamous cell carcinoma cases from the indigenous population of Karachi

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Objectives: To evaluate conventional clinical and prognostic variables in Oral Squamous Cell Carcinoma (OSCC) cases from the indigenous population of Karachi.

Methods: Common clinical and pathological parameters related to poor prognosis of 150 OSCC cases presented at two major treatment facilities of Karachi were evaluated. The reporting included demographic details and variables like intra-oral subsites, clinical stage and histological grades. Recurrence of tumor after initial resection was also documented.

Results: The patient's population comprised of 98 males and 52 females. The mean age was 47.1 + 12.22 (range: 20–78 years). Maximum numbers were seen in the 41–50 years age group. Urdu-speaking community was the most affected ethnic group (n=75). Histopathological analysis revealed that majority of cases was moderately differentiated tumors (59%) with clinical stage II (35%) or IV (29%). The most common intra-oral subsite came out to be buccal mucosa of cheeks (56%) followed by lateral borders of tongue (21%), lips (13%), alveolar (6%), palate (2.6%) floor of mouth (1.3%), etc. Recurrence was observed in 8 out of 150 cases. All patients underwent primary resection ± neck dissection and reconstruction where possible.

Conclusions: Overall experience with oral squamous cell carcinoma shows that it has a high tendency for local invasion as well as dissemination to regional lymph nodes, i.e. cervical lymph nodes, both are associated with a poor prognosis. Preventable risk factor of tobacco chewing has been observed in majority of these cases.

Poster Presentations.

Role of muscarinic G-protein coupled receptor (GPCR) in prostate cancer growth and survival

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Introduction: Despite advances in early detection and treatment, prostate cancer remains one of the most frequently diagnosed cancers and a leading cause of cancer death in males worldwide. Much work has been done on androgen receptors in relation to proliferation and survival, and it has been shown that cancers of this organ usually turn androgen insensitive and

resistant to therapy. There has been very little, if any, work done on the precise contribution of cell surface muscarinic GPCRs in prostate cancer growth.

Methods: Here we determined the role of the M1 receptor in prostate cancer cell proliferation. The treatment of PC-3 cells with selective M1 receptor antagonists, Dicyclomine exhibited anti-proliferative effects using growth assays, while M1 receptor agonist, Pilocarpine exhibited increased proliferation compared with the untreated cells.

Results and Conclusions: Further studies will dissect molecular pathways to determine the role of the M1 receptor in androgen sensitive and androgen insensitive prostate cancer cell growth and proliferation. The results of this work may help inform clinical and public health interventions for prostate cancer by providing insights into the biology of prostate cancer progression.

Co and contra-regulation of glycosylated proteins in three distinct regions of schizophrenics brain

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Introduction and objectives: Schizophrenia, a multifactorial disorder exhibits diverse neuropathological aberrations with altered protein expression and post translational modifications as a surfacing evidence that may also contribute to its pathophysiology. To investigate the integrated picture of molecular changes and potential alterations in expression of specific glycosylated proteins in different brain regions compared to physiologically normal brains. We aimed to provide more holistic view of three brain regions; and their dynamic cross-talk providing insights into the underlying molecular mechanisms associated with schizophrenia.

Methods: Present study entails, differential proteomic analysis of autopsied brain regions of schizophrenic; substantia nigra, cortex, and hippocampus (n=7 each), by using sodium dodecyl sulphate polyacrylamide gel electrophoresis coupled with immunoblot and DIG (digoxigenin) labelling followed by ESI-QTOF MS analysis for validation.

Results: We have identified fourteen glycosylated protein components with altered expression among the brain regions. The 50KDa (Glial fibrillary acidic protein, GFAP) and 84KDa (mitochondrial inner membrane protein) are contra-regulated between substantia nigra and cortex. While T-complex protein 1 subunit zeta of 58KDa is co-regulated between substantia nigra and hippocampus. The remaining five proteins identified with significantly altered glycosylation intensity in the specific brain regions give evidence of their explicit regional function. Additionally, nine proteins commonly appeared non-glycosylated in the three studied brain regions.

Expression and association of CDK10 with ETS2 during human corneal wound healing

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Introduction and objectives: Corneal related complications are major health concerns worldwide because its progression is associated with significant impaired vision. Therefore, there is an urgent need to develop reliable understanding of the underlying mechanism of corneal epithelial wound healing to apply therapeutic options. We aimed to investigate the alterations in protein expression and association of CDK10 and ETS2 during corneal epithelial migration and to demonstrate the networks of the total identified proteins with potential dual functions.

Methods: In this study, human corneal epithelial cells lines (HCEC) have been used for wound healing model. Mechanical wound was made in HCEC lines and healing was monitored at 24,

48 and 72 h of post wounding. Epithelium was scrapped at 24, 48 and 72 h, followed by protein quantification using BCA kit. The wounded and unwounded cells were subjected to SDS-PAGE and two-dimensional electrophoresis (2DE). Mass Spectrometry (MALDI TOF) was done to identify the proteins through protein database searches. The identified protein were further analyzed and validated by western blot analysis. A further insight into the links among the identified proteins and their functional roles were analyzed by STRING 8.3 software.

Results: A significant finding of the present study is the identification of Cdk10, EFN3, RAB 34, RRAS, HSP2 and HSP90 in healing corneal epithelium at active phase of migration. Expression of CDK10 and ETS2 were validated using antibody by western blot. Interaction association network analysis further confirms the close interacting relationship between CDK10 and ETS2 proteins.

Conclusions: These findings are one step forward in identifying the mechanism of wound repair or re-epithelialization. Association of CDK10 and ETS2 in migrating samples suggested that they have positively regulated the cell cycle and cell proliferation and hence mediate the wound healing. This study may also increase the understanding of normal and abnormal corneal function with likely relevance to corneal disease and transplants.

Functional association of bad and 14-3-3 proteins in schizophrenic patients

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Introduction and objectives: Schizophrenia is a multifactorial disease which involves the exogenous and endogenous interaction and is characterized by neuronal reduction, dendrite deficits, decreased synaptic markers and alteration in synaptic circuitry. Schizophrenia is a neurodevelopmental disorder but apoptosis may contribute to the pathophysiology of schizophrenia due to progressive clinical deterioration and subtle neurostructural changes following the onset of psychosis. Apoptosis is a mechanism of cell death, is regulated by a complex cascade of pro and anti-apoptotic proteins. Dysregulation of apoptosis in several cortical regions of schizophrenia indicates that apoptotic vulnerability is increased. Pro-apoptotic protein BAD (Bcl-2 associated death promoter) interacts with anti-apoptotic BCL-2 and inhibit its activity while 14-3-3s binds with BAD and inhibit apoptosis that it act as an ant apoptotic protein. Various proteomic based studies shown reduction of 14-3-3 proteins in schizophrenic brains. In this study we aimed to identify and validate the differential expression of BAD and 14-3-3s by western blotting and their association network with other proteins by String 10, Qiagen pathway data bases.

Methods: Expression of BAD and 14-3-3 proteins from autopsied schizophrenic brain cortex were validated by western blotting and In Silico analysis was done to see the association with other proteins by using String database, Qiagen pathway.

Results: We have identified differential expression of BAD and 14-3-3 proteins in cortex region of schizophrenic human brain by western blotting. Interaction association network between BAD and 14-3-3 (YWHAQ, YWHAH, YWHAE, YWHAB, YWHAZ, YWHAG) was analyzed by string software. Qiagen pathway further confirms the close interacting relationship between BAD and 14-3-3 proteins in apoptotic pathway related to schizophrenic patients.

Conclusions: Apoptotic proteins BAD and 14-3-3s association in Schizophrenic brain cortex region provide insight into the apoptotic pathways which play role in the pathophysiology of schizophrenia which could lead to development of therapeutic drugs to target apoptotic proteins and their interacting partners.

Kisspeptin and unexplained infertility

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Introduction and Objectives: To compare Kisspeptin (KP) levels in females with infertility to male, female and unexplained causes and associate it with success of treatment after ICSI.

Methods: Cross sectional study carried from August 2014 till May 2015 included all categories of infertility with duration > two years, female age between 20 and 50 years and body mass index > 18 < 35 kg/m². Down-regulation of ovaries was followed by calculated stimulation, ovulation induction, oocytes retrieval, ICSI, in vitro maturation of embryos and finally blastocysts transfer. KP levels were measured by enzyme linked immuno sorbent assay on day of ovulation induction. Failure of procedure was detected by beta human chorionic gonadotropin < 5-25mIU/ml (non-pregnant) whereas females with levels > 25mIU/ml and no cardiac activity had preclinical abortions. Clinical pregnancy group had confirmed cardiac activity on trans vaginal scan. Data was analyzed using SPSS 15.

Results: Females who completed the procedure (168) were stratified; 40 (24%), 66 (39%) and 62 (37%) with male, female and unexplained infertility. The KP levels were 285.38 ± 23.96 ng/L (mean ± SD). The females with unexplained infertility had lowest KP (101.4 ± 11.96) which was statistically significant (p < 0.05). The results declared 66 (39%) females as non-pregnant, 30 (18%) with preclinical abortions and 72 (43%) with clinical pregnancies. Out of non-pregnant females 42 (64%) had unexplained cause of infertility.

Conclusions: Low KP levels in unexplained females explains the peripheral role of the neuropeptide, deficiency of e=which leads to failure of implantation after ICSI.

Reliability of nephrin as an early diagnostic biomarker for screening diabetic nephropathy

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Introduction and objectives: Diabetic nephropathy occurs as a result of proximal tubule dysfunction with podocyte damage due to increased advanced glycation end-products insults in diabetes. Nephrin, one of the three proteins that make up the podocyte architecture, is excreted foremost following renal damage. The aim of this study was to evaluate the efficiency and reliability of nephrin for an early biomarker of kidney damage in diabetic patients.

Methods: Urine samples (78) were collected from diabetic center. Protein and glucose were determined by Dipstick. The patients were grouped on the basis of Albumin/creatinine ratio (UACR) as normoalbuminuric, microalbuminuric and macroalbuminuric with UACR less than 30 mg/g, from 30 to 300 mg/g, and above 300 mg/g respectively. ELISA (Exocell USA) was used for Nephrin estimation. Statistical evaluation was done.

Results: Nephriuria was present in 70 (89.7%) out of 78 diabetic patients including 35 (81.4%) normoalbuminurics, 5 (6.4%) micro-albuminurics and all 5 of macroalbuminurics (p < 0.027). When associated with duration of diabetes, 21 (91.3%) positive with less than three years of diabetes, 24 (92.3%) with three to seven years and 25 (86.2%) in more than seven years (p < 0.039). Nephrin levels were found increasing from Normo-(0.86 µg/ml) to Micro-(11.6 µg/ml) to Macroalbuminuria group (47.6 µg/ml), compared to 0.15 µg/ml in comparison group.

Conclusions: The increase in nephrin levels from 0.86 µg/ml in patients with normal albuminuria to 47.6 µg/ml in patients with macroalbuminuria suggests that Nephrin precedes albumin in urine predicting early signs of kidney damage.

Bioinformatics navigation predicted structure-function dynamics of Calpain10 isoforms

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Introduction and objectives: Calpain10, a member of cysteine proteinase family, is present in eight different isoforms (a-h) exact mechanism through which it influences the insulin secretion and action is scanty.

Methods: We have applied bioinformatics approach to envisage its mechanism of action and possible involvement in diseases. National Center for Biotechnology Information Blast was used for humans' nucleotide and protein sequence alignments of the calpain10 isoforms a, c and g. Further, NCBI conserved domain tool and CDART were used for other species.

Results: Results of blast showed 100% nucleotide whereas 91% and 97% amino acid sequence identity among isoforms of Homo sapiens' calpain10 c and g with calpain10 a. There was 82% query coverage of calpain10 c and 17% of calpain10 g with calpain10 a. Conserved domain analyses of peptide sequences indicated domains IIa and IIb have significant homology with the catalytic domain of the cysteine protease superfamily in all three isoforms. The domain III and IV of isoform a and domain III of isoform c were found homologous with linker C2 like subdomain III of mu-calpain.

Conclusion: It is predicted that calpain10 a and c isoforms may have a role in developing diseases such as neurodegenerative, cardio-vascular, cataract, osteopenia and cancer. Whereas isoform g may exhibit protease activities having papa-in-like domain only.

TP53 protein overexpression in oral squamous cell carcinoma (OSCC): correlation with histologic variables and 5 years survival in Pakistani patients

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Introduction and objectives: Alteration of TP53 gene in oral squamous cell carcinoma (OSCC) is believed to be associated with reduced overall survival (OS), the 5 year prognosis in our study and the disease-free survival (DFS). The aim of this study is to determine whether TP53 protein over expression in OSCC is a prognostic indicator of survival in Pakistani cancer patients along with its correlation with risk factors including smoking, chewing habits, histological variables like grade and stage of the tumor in a high risk population.

Methods: A total of 140 patients of OSCC were part of our study. TP53 protein over expression was investigated by means of immunohistochemistry.

Results Overexpression of p53 protein was observed in 75 patients (54%) using a threshold of 10% stained tumor nuclei. Patients with p53 negative tumors had improved OS when compared with patients with p53 positive tumors. This difference was statistically significant ($p=0.036$) in univariate Cox regression analysis however, it lost its worth in the multivariate analysis.

Conclusion: This works supports that patients with p53 overexpression had a significantly poor overall survival compared to p53 negative patients. However, p53 overexpression was not associated with patient's disease free survival.

Cardiovascular effects of aqueous-methanolic extract of bergenia ligulata in experimental animals

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Introduction and objectives: Prevalence of hypertension, a major risk factor for diseases like ischemic heart disease and cerebrovascular stroke, is on the rise. The aim of this study was to explore the cardiovascular effects of the aqueous-methanolic extract of *Bergenia Ligulata* rhizome (Bl·Cr), to provide pharmacological basis for its medicinal use in hypertension.

Methods: Aortae from Sprague-Dawley rats and atria from guinea-pigs were placed in 5 ml and 15 ml isolated tissue bath assemblies respectively, which were maintained at 37 °C with constant carbogen supply, filled with physiological salt solution (Kreb's solution) and connected to a force transducer and Power-Lab attached with a computer.

Results: Bl·Cr, in isolated guinea-pig atria, equally inhibited force and rate of spontaneous atrial contractions. When tested on phenylephrine (PE, 1 μM) and K⁺(80 mM)-induced vasoconstriction, Bl·Cr caused a concentration-dependent relaxation and also caused a suppression of PE (1 μM) control peaks in Ca²⁺-free medium.

Conclusions: These data indicate that Bl·Cr exhibits cardio-suppressant and vaso-dilatory properties. The vasodilator effect of the plant extract is mediated through inhibition of Ca⁺⁺ influx via membranous Ca⁺⁺ channels as well as Ca⁺⁺ release from intracellular stores. Further studies are required to elaborate the anti-hypertensive activity of the plant.

Association of Hepatitis G with liver dysfunction in treatment responders Hepatitis C patients

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Background: About 10 million Pakistani population is infected with Hepatitis C virus (HCV). The prevalence is even more pronounced among high risk population. Combination of standard interferon and ribavirin is still the first line therapy with sustained viral response (SVR) up to 40–50%. Pool of relapsers and non-responders is increasing in Pakistan and needs treatment with pegylated interferon plus ribavirin (peg IFN/RIB). The hepatitis G virus (HGV), a recently identified member of the Flaviviridae family, can cause chronic infection in man.

Methods: 250 treatment responders Hepatitis C patients (undetectable HCV RNA in the serum after 24 weeks of post treatment follow up) were recruited from Jinnah Post Medical College and Ziauddin Hospital.

Liver function tests of all patients were assessed by automatic analyzer kits. HGV RNA was investigated in serum samples by reverse transcription and polymerase chain reaction amplification of the 5' non-coding region of HCV and hybridization to a specific probe. The liver function tests of HGV RNA sero-positive and sero-negative patients were compared.

Results: A total of 250 treatment responder hepatitis C patients were evaluated with a mean age 44 ± 5.55 . Males constituted 38.4% (N=96) while female constituted 61.6% (N=154) of our study group. Mean total bilirubin, direct bilirubin and indirect bilirubin was 0.772 ± 0.19 mg/dl, 0.308 ± 0.99 mg/dl and 0.496 ± 0.55 mg/dl respectively. Mean ALT was 81.97 ± 38.03 units/L and total protein was g/dl 5.25 ± 0.44 . Statically significant difference of mean LFTs was found between hepatitis G positive and negative patients with p -value = 0.001. To find out association of LFTs with hepatitis G positive patients, chi-square was used. Total bilirubin, direct bilirubin, indirect bilirubin, AST, GGT, and APTT are found to be associated with hepatitis G with p -value of 0.001. By Log Regression we found significant and positive association of Hepatitis G with Total bilirubin, direct bilirubin, indirect bilirubin, GGT and APTT (p -value = 0.001 at 95% CI).

Conclusions: Liver dysfunction in treatment responder hepatitis C patients is found to be associated with novel Hepatitis G virus.

Combination therapy of non-steroidal anti-inflammatory drugs and anti-oxidants: An effective regimen for inflammatory disorders?

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Introduction and Objectives: Non-steroidal anti-inflammatory drugs are of therapeutic value for inflammatory disorders. Reactive Oxygen Species may initiate and/or aggravate inflammation. Information regarding the interaction of vitamin E with NSAIDs in inflammation is not well documented and is controversial.

Methods: This study was conducted to determine the effect of aspirin with vitamin E supplementation in inflammation, and to explore the possible interactions of vitamin E with one drug from each sub group of NSAIDs with respect to their anti-inflammatory activity using animal model of inflammation, DNA degradation and lipid peroxidation assays.

Results: Our results showed that vitamin E caused 10% inhibition in edema at 400 mg/kg dose while aspirin elicited complete inhibition in edema at 200 mg/kg dose. Co-administration of different doses of aspirin with vitamin E showed a dose-dependent increase in edema inhibition. At 100 mg/kg aspirin showed ~50% inhibition in edema. While the same dose of aspirin with vitamin E supplementation (200 mg/kg) showed a significant increase (~25%) in its anti-inflammatory effect. Similar results were obtained with DNA degradation and lipid peroxidation assays.

Conclusions: In conclusion, aspirin, diclofenac sodium, and celecoxib, with vitamin E supplementation, showed significant synergistic effects that could be used as an effective preventive and therapeutic regimen for inflammatory diseases.

Clinical efficiency and cost effectiveness of macroprolactin screening in hyperprolactinemic patients

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Introduction and Objectives: Macroprolactin (MaPRL) is a biologically inactive compound which leads to falsely elevated prolactin levels. It is recommended that all sera with increased total prolactin concentrations be sub fractionated by PEG precipitation to measure the bioactive monomeric prolactin concentration to prevent misdiagnosis and unnecessary investigations. We aim to determine clinical efficiency and cost effectiveness of PEG screening of hyperprolactinemic sera.

Methods: In this Retrospective cross sectional study patients with high total prolactin levels were screened by PEG precipitation. Relevant diagnosis of Macroprolactinemia and True Hyperprolactinemia was made based on their absolute PEG treated monomeric prolactin level. They were then contacted on phone and a detailed history of their clinical symptoms along with their radiological workups was inquired.

Results: Frequency of macroprolactin was 60.7% seen in 145 patients and true hyperprolactinemia was observed in 94 (39.3%) patients. More asymptomatic patients were reported in the macroprolactin as compared to true hyperprolactinemic group ($p \leq 0.05$). 37 (39.4%) patients with true hyperprolactinemia had further radiological investigations done where-as only 8 (5.5%) of the patients with macroprolactin had undergone further radiological workups. The total use of resources in the true hyperprolactinemic group was significantly higher, 943000 PKR (10031 PKR per person) vs 418450 PKR (2884 PKR per person) ($p \leq 0.05$).

Retinal nerve fiber layer thickness in a subset of Karachi (Pakistan) population

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Introduction and objectives: To provide the normal range of retinal nerve fiber layer (RNFL) thickness in a subset of Karachi population by Spectralis OCT and to evaluate the effects of age and

gender on it.

Methods: 300 eyes from 150 healthy subjects aged 40 years and above with no ocular pathologies were examined using standard protocols by a single examiner. Subjects with history of diabetic or hypertensive retinopathy, raised intraocular pressure (> 21 mmHg) and previous intraocular or laser surgery were excluded from the study. The mean retinal nerve fiber layer thickness was calculated and was correlated with age and gender difference.

Results: The mean global retinal nerve fiber layer thickness was found to be $99.02 \pm 9.08 \mu\text{m}$ in our set of population. Out of four quadrants the maximum RNFL thickness was found in inferior quadrant ($126.45 \pm 16.23 \mu\text{m}$) followed by the thickness of $121.50 \pm 15.03 \mu\text{m}$ in superior quadrant, $70 \pm 14 \mu\text{m}$ in nasal quadrant and $68.90 \pm 13.10 \mu\text{m}$ in temporal quadrant. We found strong negative correlation of RNFL thickness with age ($P \leq 0.001$) and not significant relation with gender ($P=0.8$).

Conclusions: Keeping in mind the variations in RNFL thickness with ethnic differences, this study provides the normal values of RNFL thickness according to our set of population. It is concluded that RNFL thickness decreases significantly with increasing age but gender had no significant effect on it.

Prevalence of metabolic syndrome in adolescents/first year medical students of a public sector medical college in Quetta, Pakistan

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Introduction and objectives: Metabolic syndrome is a disorder of energy utilization and storage. According to the International Diabetes Federation, metabolic syndrome in adolescents more than 16 years is defined as “the presence in an individual of central obesity plus any two of the following four risk factors: hypertriglyceridemia, hypertension, low high density lipoprotein and high fasting glucose levels.

Reports suggest that around 25% of the world’s adult population suffers from metabolic syndrome. People with metabolic syndrome are twice as likely to die and have three times higher chances of heart attack or stroke.

The study is designed to determine the prevalence of metabolic syndrome in first year medical students between 17 and 19 years, of a public sector medical college in Quetta.

Methods: A Cross-sectional study was conducted on all the healthy first year medical students, ages from 17 to 19 years studying in a public sector medical college in Quetta, Pakistan. Demographic data were assessed by using self-administered questionnaire. Height, weight and waist circumference were measured along with fasting blood sugar, high density lipoprotein and triglycerides by using Automated Biochemistry Analyzer. Analysis was done on SPSS 20. Descriptive statistics was used to present the data and logistic regression was applied to see the relationship between the study variables ($P < 0.05$).

Results: A total of 225 were included in the study, out of which ($n=127$, 56.4%) were females. Mean age was 18.79 ± 0.38 years. Prevalence of metabolic syndrome was found to be 14.2% in our study sample. Increased waist circumference, fasting blood sugar, triglycerides were associated with an increased likelihood of exhibiting metabolic syndrome. Male gender and high HDL were found to be protective against metabolic syndrome.

Conclusion: Prevalence of metabolic syndrome is increasing in our adolescent population which demands screening from childhood and early adolescence. Early screening, identification and lifestyle interventions will decrease the morbidity from chronic illnesses like Coronary artery disease and Diabetes mellitus.

Association of vitamin D with outcome after intra cytoplasmic sperm injection

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Introduction and objectives: To observe effects of vitamin D levels on pregnancy outcome after intra cytoplasmic sperm injection (ICSI).

Methods: It was a cross sectional study conducted in Australian Concept Infertility Medical Center from July 2011 to August 2014. Estimation of 25 hydroxy cholecalciferol (25-OHD) of consented females (252) was done before treatment protocol for ICSI. Results of β hCG performed 14 days after embryo transfer categorized groups; Pregnant with β hCG more than 25 IU/ml and rest included in non-pregnant group. Both groups were compared by independent sample *t*-test and Pearson Chi Square test. Binary Logistic Regression Analysis was used to estimate odds ratio of pregnancy outcome with its predictors.

Results: The mean value of 25-OHD, number of oocytes, fertilized oocytes and endometrial thickness was significantly higher in pregnant women. A significant positive association of 25-OHD with clinical pregnancy and thickness of endometrium was observed. After adjustment with female age and BMI positive association of vitamin D with endometrial thickness was observed.

Conclusion: Deficiency of (25-OH) in females hinders the accomplishment of optimal endometrial thickness required for implantation of embryo after ICSI. The improvement in vitamin D status can thus improve success results in assisted reproductive clinics.

Comparison clinical outcome and cost-effectiveness of Tegafer and Capecitabine for the treatment of metastatic colorectal cancer

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Introduction and objectives: The development of numerous oral chemotherapy agents has led to a new paradigm in management of cancer. Treatment modalities with cost effective approach and better clinical outcomes can be proved to be an effective strategy to reduce disease burden.

To evaluate the clinical and cost effectiveness of oral chemotherapeutic agents Capecitabine and Tegafer as first-line treatments for patients with metastatic colorectal cancer.

Methods: The analysis involved 121 patients of metastatic colonic carcinoma admitted at Almehr Tibi Amdad Hospice care, Karachi between September 2013 to March 2015. The patients selected to enrolled in present study were aged ≥ 18 years, both sex and histologically diagnosed cases of colorectal cancer (stage 3 with primary surgical resection. While those with history of hypersensitivity to Tegafer, fluoropyrimidines, capecitabine, or any other ingredients of this product, Inadequate hematopoietic function WBC 4000/mm³; ANC 2000/mm³; Platelet 100,000/mm³, Inadequate organ function, CNS metastasis, life expectancy less than 3 months and those who were not willing to participate were excluded. All subjects fulfilling the eligibility criteria were randomly assigned to two groups, one group received oral capecitabine (1250 mg/m² twice daily for 14 days followed by one week gap) and other received Capsule Tegafer 500 mg daily for 28 days followed by one week gap. All the patients were followed and response was observed after 3 months, 6 months and at the end of year.

Results: Patients receiving Tegafer showed significantly less incidence of alopecia, diarrhea and stomatitis ($P < 0.05$) while incidence of hand-foot syndrome and grade 3/4 hyperbilirubinemia were found to be significantly more as compared to capecitabine ($P < 0.0001$). Cost of treatment per patient and per cycle using oral Tegafer was less than that using capecitabine.

Conclusion: Tegafer showed overall improved response rates, cost effectiveness and has lesser side effects in comparison with Capecitabine.

Radiological study of the mean age of fusion of medial end of the clavicle as a parameter of age

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Introduction and Objectives: Estimation of the age of a person is a problem that a forensic expert confronts in both living and dead subjects. 21 years of age is of immense medico-legal importance, as it entitles a person to be employed as a gazetted officer, getting driving license for all types of vehicles, participation in various sports events and is a mandatory parameter for identification in dead. This study gives us an authentic criterion for assessing 21 years age, by using closure of ossification centers at medial clavicular epiphysis by applying radiological method for subjects living in Karachi.

Methods: The main objective of this study is to determine the mean age of fusion of medial end of clavicle by radiological method, to get a perfect estimator of 21 years of age.

Results: The mean age of fusion of medial end of the clavicle was found to be 21 ± 1.43 years. Significant difference was observed in male to female ratio 21.14 ± 1.41 versus 20.65 ± 1.94 (P value < 0.05). Similarly, statistically significant difference was observed between lower class to middle class and higher class of all society. No difference was observed between the various ethnic groups.

Conclusion: Socio-economic factors such as diet and nutrition directly affects bone growth and hence bone age. The results of fusion of the medial end of the clavicle are not affected by ethnicity. More studies should be conducted in various parts of country to make a natural standard in setting up uniform criteria for assessing age at or above 21 years.

Centrathrum anthelminticum (kalizeri) minimize the risk of chemically-induced hepatotoxicity in rats.

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Introduction and Objectives: Carbon tetrachloride (CCl₄) is a cleaning agent used in industry. Its vapours are injurious for many body tissues especially liver. The study was designed to evaluate the hepatoprotective effect of ethanolic seeds extract (ESeT) of *Centrathrum anthelminticum* (kali zeri) in CCl₄-induced hepatotoxic rats.

Methods: Rats were made hepatotoxic by intraperitoneal injection of CCl₄ (3 ml/kg) on 3rd and 5th day of trial and divided into hepatotoxic control (distilled water 1 ml/kg), positive control (silymarin 100 mg/kg), and test (ESeT 800 mg/kg) groups. Normal control rats were also run. After 24 h of last dose of CCl₄, body weights (BW) of all rats were recorded, sacrificed them to collect blood and serum to analyze liver-specific parameters. In addition, livers were dissected out carefully to estimate lipid peroxidation (LPO).

Results: ESeT (600 mg) not only significantly decreased the percent reduction in BW of test rats but also improved the levels of alanine transferase (ALT), aspartate transferase (AST), alkaline phosphates (ALP), total bilirubin, total protein and albumin. Beside these, percent inhibition of LPO was also increased.

Conclusion: The ESeT of *Centrathrum anthelminticum* was found as a hepatoprotective and antioxidant agent that could be used for the preparation of medicine in future.

Spectrum of preneoplastic and neoplastic lesions of intestine in a tertiary care hospital of Karachi, Pakistan

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Introduction: Gastrointestinal malignancies are the global oncological problems. It is therefore imperative to investigate the spectrum of this pathology in Pakistan.

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

Methods: The study was conducted during 2009–2014. All the cases of preneoplastic and neoplastic lesions of intestine received during the period of 6 years were reviewed. The data was subjected to statistical analysis using SPSS version 22.

Results: A total of 64 cases were diagnosed as premalignant lesions of intestine consisting of ulcerative colitis (19/29.7%), adenomatous polyp (17/26.6%), dysplasia (14/21.9%) and adenoma (14/21.9%).

About 478 cases were diagnosed as malignant lesions of intestine as:

- i. Adenocarcinoma grade I (65/13.6%), grade II (283/52.2%), grade III (95/19.9%)
- ii. Squamous cell carcinoma grade I (2/0.4%), grade II (7/1.5%), grade III (3/0.8%)
- iii. Metastatic adenocarcinoma (19/4.0%)
- iv. Neuroendocrine (4/0.8%)

Conclusion: Our study showed ulcerative colitis as the commonest premalignant lesion and grade II adenocarcinoma the most common malignancy of intestine.

Comparison of antibacterial activity of Punica granatum and Syzyium cumini leaf extracts.

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Introduction and objectives: Plants are the richest source of phytochemicals, not only protecting the plants itself but also benefits the mankind. Punica granatum commonly known as pomegranate and Syzyium cumini known as Jamun are well known for its phenolic compounds having antioxidant potential. They are used in traditional medicine for the treatment of diabetes, diarrheal diseases, antiparasitic and antibacterial infections.

Methods: The aim of the study was to identify the antimicrobial potential of two leaf extracts of Punica granatum and Syzyium cumini. fresh leaves were washed and ground with distilled water. The extract was obtained which was filtered by Whatman #1 filter for the removal of particles. The antimicrobial activity was performed by agar well diffusion method against E.coli, Bacillus subtilis, Klebsiella oxytoca, Salmonella typhi, Micrococcus leutus, Proteus mirabilis, Staph aureus and Candida albicans. PBS was taken as negative while streptomycin as positive control.

Results: It was found that the extract of Punica granatum was inhibitory to all microorganisms. The highest zone of inhibition was observed against Proteus mirabilis and E.coli (25 mm). Leaf extract of Syzyium cumini do not show any antimicrobial activity.

Conclusions: Pomegranate leaf extract can be effective against GIT infections. Jamun, as reported earlier, is well known to control diabetes cannot be used to control infections.

Identification, differentiation and sensitivity pattern of Haemophilus influenzae and Haemophilus parainfluenzae

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Introduction and objectives: H.influenzae is a pleomorphic Gram-negative coccobacillus that is isolated predominantly from the respiratory tract and this opportunistic species may cause

systemic infections such as epiglottitis, meningitis, bacteremia/sepsis, bronchitis and chronic obstructive pulmonary disease and otitis in young children. H. parainfluenzae, in contrast, is a saprophyte that colonizes the upper respiratory tract but it hardly causes respiratory tract infections and only occasionally infectious endocarditis. But many strains are now becoming resistant to beta lactam antibiotics. We aim to test the sensitivity pattern of Haemophilus influenzae and Haemophilus parainfluenzae.

Methods: Total 45 sputum samples were analyzed collected from different hospitalized patients. Specimens were inoculated on blood agar plates and incubated in 5-% CO₂ at 37 °C for 24–72 h. Gram stain were performed for direct examination and differentiation was done on the basis of availability of hemin(X) and NAD (V) factor, oxidase and slide agglutination test. Antibiogram was done by CLSI method. Statistical analysis was also performed using SPSS version 17.

Results: H.parainfluenzae showed such sensitivity pattern AMP75%, AMC80%, CRO80%, SXT42%, C92%, CIP/OFX84% while H. influenzae showed 100% sensitivity towards AMP, AMC, CRO, SXT, C and 92% sensitivity against CIP.

Conclusions: Proper diagnosis and selective use of antibiotics can minimize the resistivity of these organisms.

Association between interleukin 6 gene polymorphism and human papilloma virus infection in oral squamous cell carcinoma patients

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Introduction and objectives: Human papilloma virus (HPV) and IL 6 gene polymorphism are proven independent risk factors for development of Oral Squamous Cell Carcinoma. The objectives of this study were to find out frequency of IL6 gene polymorphism and HPV and to evaluate any association between HPV and IL6 gene polymorphism in OSCC patients and its effect on disease prognosis.

Methods: In this cross-sectional study 140 OSCC patients (104 males and 36 females), aged 18yrs and above were selected. Detailed questionnaire was filled followed by sample collection. After DNA extraction PCR analysis for HPV and RFLP for IL6 gene polymorphism was carried out.

Results: Mean age of the patients was 43.5 ± 11.84 years (range 31–40 years). Majority (n=45; 32.1%) belonged to Urdu speaking ethnic group and were habitual eaters of Pan (n=87; 62.1%) and Gutka (n=82; 58.6%). The most common site of OSCC was buccal mucosa (61.4%). Most of the patients presented with grade II (55%) and late stages (stage III & IV) (55.7%) of OSCC. Out of 140 samples, 12 (8.6%) tested positive for HPV gene with the following pattern of IL 6 gene polymorphism; GG (46.4%), GC (39.3%), CC (14.3%). A significant association was observed between stages III & IV of OSCC and IL6 genotypes GC (P=0.001) and CC (P=0.002). Also strong positive association (P=0.003) was found between HPV and IL 6 (P=0.007) CC homozygote genotype.

Conclusions: This study finds an association between HPV and IL6 gene polymorphism in late stage OSCC patients suggesting rapid and aggressive progress of oral carcinogenesis.

Comparison of virulence factors of isolated Candida spp from lower respiratory tract infections in hospitalized patients

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Introduction and objectives: Candida a diverse group of fungus has bacterial and fungal properties and 80% commensals of normal human body i.e C. albicans and C. tropicalis are involved in various diseases like oral thrush, vulvovaginal candidiasis etc. when they get opportunity and they now are considered as the third most pathogenic strain causing various diseases in human. The objective is to compare the virulence properties of different

Candida spp.

Methods: 126 sputum samples were collected from May to August 2015 from hospitalized patients. Samples were inoculated on SDA. Identification was processed by gram staining, germ tube test, Chlamyospore detection and CHROM agar. Phospholipase, proteinase and hemolytic activity were deliberated in them. Statistical analysis was also performed using SPSS version 17.

Results: 51% males and 48% female were found to be infected with *Candida* spp. The most prevalent organism was *C. albicans* (87.5%) followed by *C. glabrata* (8.56%) and *C. tropicalis* (3.08%). Phospholipase activity (45.9%), proteinase (30.1%) and haemolysin activity (19.9%) was shown by *C. albicans*. Phospholipase and proteinase activity was also observed in *C. tropicalis* but not in *C. glabrata*.

Conclusions: Present study indicate that *C. albicans* being a member of normal flora pose a major threat in immune-compromised patients and majors should be enforced to prevent infections with *Candida*.

Structural investigation of Neisseria meningitidis pathogenic factors; a key step towards drug designing

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Introduction and objectives: The Gram negative human pathogenic bacterium *Neisseria meningitidis* is responsible for causing meningitis and septicemia worldwide. A substantive question in bioinformatics analysis of bacterial genome is to ascribe a three dimensional structure as well as a biologic role to all the coding regions. Here we performed structural bioinformatics analyses of important *Neisseria meningitidis* pathogenic factors involved in protein biosynthesis including methionyl-tRNA synthetase, 16SRNA methyltransferase, translation elongation factor-Tu and putative RNA methylase.

Methods: Homology modeling of these important drug targets was carried out with better templates by MODELLER software and evaluated by Prosa and Procheck standalone softwares.

Results: The study provided detailed structures of important proteins required for the pathogenesis of this organism.

Conclusions: In future, these investigations will help in receptor-based drug designing against meningococcal infections.

Evaluation of antimicrobial activity of Aloe vera.

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Introduction and objectives: Aloe vera is a cactus, belongs to the family Liliaceae, and is well known for its use as traditional medicine. It is used for gastrointestinal disorders, stimulates body's immune response, for treatment of burns, eczema, psoriasis, to heal skin infections. Aloe vera is known to hold therapeutic properties due to bioactive components present in it for both infectious and non-infectious diseases.

Methods: Antimicrobial activity of Aloe vera was investigated against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Micrococcus*, *Klebsiella pneumoniae*, *Proteus*, *Staphylococcus aureus*, *Salmonella typhi* and *Candida albicans*. The organisms were isolated from clinical samples and identified by conventional methods. Susceptibility test was carried out by agar well diffusion method. Aloe vera used as methanolic extract and pure gel. All experiments were performed in triplicate.

Results: Zone of inhibition was observed in all forms of Aloe vera. The maximum zone of inhibition was found in *E. coli* and followed by *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. aureus* and *Bacillus subtilis* showed least zone of inhibition. It was also found that inhibitory affect against *Candida albicans* was also maximum.

Conclusions: The result of this study would tend to give

credence to the use of Aloe vera for the formulation of certain compounds that would coin new, effective and more potent antimicrobial drugs to combat pathogenic microorganisms.

Bioinformatics analysis of histone-lysine N-methyltransferase - SETD1A gene in association with schizophrenia

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Bioinformatics methodologies have made it possible to profile the global composition of tissue or organelle at specific time point or under particular developmental or disease state. A genetic variant of SETD1A has yielded significant genome-wide association with schizophrenia, suggesting that this SETD1A plays a key role in its etiology. The SETD1A gene plays a part in chromatin modification - an important cellular process that reduces the size of DNA so it can fit inside a cell and regulate gene expression. It encodes a catalytic subunit of an enzyme that adds methyl groups to histone proteins - genes for transcription.

The present study is based on computational analysis of SETD1A gene exploring molecular networks of interacting targets by using advanced bioinformatics tools such as, STRING's, KEGG, Reactome and BioGrid databases that may provide insights into the biological processes underlying schizophrenia.

Frequency of Hepatitis B and C in different area of catchment center in Gadap Town, Karachi, Pakistan.

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Introduction and objectives: Frequency of Hepatitis B Surface Antigen (HBs Ag) and Hepatitis C Virus Antibodies (anti-HCV) among population of different catchment center in Gadap area with their low social economic status. We aim to study the frequency of Hepatitis B Surface Antigen (HBs Ag) and Hepatitis C Virus Antibodies (Anti-HCV) in different catchment center of Gadap town in 12 kilometer from Baqai Medical University. The design of study will be cross sectional descriptive study. It was held in the Pathological and Molecular Laboratories, Karachi, during the period October 2011 to May 2012.

Methods: A total of 496 samples were collected. Serum was tested for Hepatitis B surface antigen and for Hepatitis C virus antibodies by ELISA. The results were subjected to chi-square analysis for determination of statistical difference between the values among different categories.

Results: Among 496 patients 91 (18.3%) were positive for HBs Ag and 171 (34.4%) were positive for Anti-HCV. The frequency of HBs Ag was seen too low as compared to anti-HCV.

Conclusions: High frequency of HCV infection needs implementation of strict screening policy for donors and public awareness campaigns about preventive measures to reduce the spread of this infection as well as other transfusion transmissible infections.

Innovative approaches in metabolomics for understanding drug resistance in breast cancer

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Introduction and objectives: Breast cancer is one of the leading cause of death worldwide. In Pakistan, prevalence of this ailment is highest amongst all types of cancer i.e. 38.5%. Major clinical setback is drug resistance in breast cancer. Metabolomics is an emerging field that utilizes information of cellular biochemistry for the early detection, diagnosis and establishment of predictive biomarkers of breast cancer. This review highlights potential metabolomic applications to pharmacology and clinical

pharmacology.

Methods: The methodology is based on inclusion exclusion criteria. Literature survey, and questionnaire were included while clinical trial was excluded. This report provides a review of 12 articles out of few were excluded.

Results: According to the survey the average response rate of a cancer drug is the lowest at 21%, suggesting that 74% of patients with cancer are over-dosed. While according to an international study, 40–50% of breast tumors will display acquired resistance.

Conclusions: When specific therapies are chosen on the basis of a patient's metabolomics profile, it will give rise to customized medicine and personalized tailored treatment. Using high-throughput information using metabolomics to clinical diagnosis and treatment can help accelerate the patient safety, quality of life and survival rate by identifying pathways involved in drug resistance.

Aberrant protein S-nitrosylation in hypertensive and diabetic-hypertensive patients: New perspective

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Hypertension and diabetes are the risk factor of morbidity and mortality. The prevalence of hypertension is expected to increase in the upcoming years. The incidence of diabetes is two fold higher in patient with hypertension. The co-existence of diabetes and hypertension causes impaired renal function, the development of diabetic retinopathy, cardiac problems, and the development of cerebral diseases. Besides these disorders hypertension and diabetes are involved in many other devastating disorders. Protein S-nitrosylation conveys a large part of the ubiquitous effect of nitric oxide on cellular signal transduction, accumulating evidence indicates important roles for S-nitrosylation both in normal physiology and in a broad spectrum of human diseases. Dysregulated S-nitrosylation has been implicated as a cause or consequence of a broad range of diseases, including asthma, cystic fibrosis, Parkinson disease, heart failure, and stroke. The role of nitrosylases and denitrosylases in governing levels of S-nitrosylation under both physiological and pathophysiological conditions is increasingly appreciated. The purpose of study is to identify s-nitrosylation in hypertensive and diabetic hypertensive serum samples. We take serum sample of hypertensive subjects (n=10), diabetic hypertensive subjects (n=10) and normotensive subjects (n=10). Total protein was quantified using Bradford assay. SDS PAGE was done after dilution of sample followed by western blotting. The results we obtained from the above mentioned methods showed that s-nitrosylation is decreased in hypertension and slightly increased in diabetic hypertension.

Structural bioinformatics analysis of transcription factors involved in Neisseria meningitidis pathogenesis; a leap towards antimeningococcal drug discovery

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Introduction and objectives: Neisseria meningitidis is a virulent pathogen causing meningitis as well as life-threatening septicemia throughout the world. Unfortunately, any vaccine or drug has not yet been developed against N.meningitidis serogroup B. Functional genomics strategies have been adapted to study the growth and pathogenesis of Neisseria meningitidis in which a library of 2850 insertional mutants was analyzed and 73 genes were identified in N.meningitidis genome responsible for causing disease.

Methods: We have selected the proteins (gene products) i.e., Dead box RNA-Helicase, Polyribonucleotide nucleotidyl-transferase PNPase and Ribonuclease-III involved in transcription for their

detailed structural analyses. These proteins are also involved in RNA-degradosome assembly.

Results: Different bioinformatics strategies were applied and the homology models of these proteins were built using protein structure-modeling program MODELLER and the models were evaluated using PROSA and PROCHECK software, as well as active sites are also predicted.

Conclusions: Since these proteins are potent drug targets, therefore their structural identification will prove to be a breakthrough in drug discovery. We hope that our study will grease the wheels for drug designing against lethal meningococcal disease.

Role of rauwolfia serpentina extract on the modulation of immobilization stress-induced behavioral deficits and adaptation to stress in rats

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Introduction: Stress is the major precipitating factor in the onset of depression, anorexia nervosa, diabetes and obesity. The goal of present study is to monitor the behavioral and biochemical effects of post stress administration of Rauwolfia serpentina extract on adaptation to immobilization stress in rats.

Methods: The plant extract (30 mg/kg) was orally administered after termination of immobilization stress daily (2 h) for 5 days to monitor any change in behavioral activities. Effects of Rauwolfia serpentina extract on immobilization stress induced deficits of food intake and body weight were determined for an understanding of the relationship between stress tolerance and behavioral changes. We also tested effects of Rauwolfia serpentina extract on endogenous leptin and glucose levels in unstressed and stressed animals to explore the possible role of HPA axis in the modulation of stress-induced behavioral deficits and adaptation to stress.

Results: The present study showed that Rauwolfia serpentina extract can blunt stress-induced anorexigenic as well as anxiogenic-like effects. Moreover, Rauwolfia serpentina extract reversed adverse effects of stress and facilitate adaptation to an uncontrollable stressor by reducing stress perception.

Conclusion: The present study shows that immobilization stress induced decreases in food intake, body weight as well as behavioral deficits were reversed by Rauwolfia serpentina extract suggesting anxiolytic like profile of drug. This effect of Rauwolfia serpentina extract can be explained in terms of additive effects of stress on serotonin (5-hydroxytryptamine; 5-HT) neurotransmission particularly via postsynaptic 5HT_{2C} as well as 5HT_{1A} receptors. It is therefore suggested that post stress administration of Rauwolfia serpentina extract provides an innovative approach for the treatment of stress related disorders.

Alteration in salivary parameters lead to oral lesions among chewable tobacco users

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Introduction: Salivary flow and composition alters under deleterious chemical irritants present in chewable tobacco. Apart from inflammation and deterioration in health of oral mucosa it can also affect the major and minor salivary glands and cause a decline in salivary flow rate. The objective of the study was to find out alteration in salivary parameters that lead to oral lesions among chewable tobacco users.

Methods: A total of 354 healthy male subjects, consuming any

form of chewable tobacco product, belonging to low socio-economic areas of Karachi were selected for this cross sectional study. A questionnaire was used to collect demographic data and details of chewing habits (using since, pack/day, duration of exposure etc.). Resting saliva of every subject was collected for 5 min and RSFR was expressed in ml/min. Salivary pH was determined by using pH strips (pH 0–14). Oral examination was done for the presence or absence of oral lesions. Data was analyzed on SPSS version 20.

Results: Out of the 354 subjects included, 27.4% consumed gutka, 24.3% niswar, 24.3% paan and 24% were multiple users. Mean Resting Salivary Flow Rate (RSFR) was 0.52 ± 0.34 ml/min, pH 6.58 ± 0.78 and 27.1% ($n=96$) had oral lesions. Highest frequency of oral lesions was found among subjects who had hypo-salivation (40%) and those having acidic pH (40%). A significant decrease in RSFR and pH and increase in frequency of oral lesions is observed with increased duration of exposure, duration of usage and increased number of tobacco packs consumed per day.

Conclusion: Increased frequency and recency of chewable tobacco use leads to decrease in RSFR and pH and hence increase in frequency of oral lesions.

Speech assessment through the tri-position articulation analysis for bilinguals (TAAB)

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Introduction: Bilingualism is regarded as a cognitive feat by today's linguists. The acquisition of more than a single language manifests the miracle of the human brain in terms of meta-linguistic (meta-phonological) skills, as it stores the form and content of multiple languages and exhibits their use in socially and linguistically appropriate contexts.

Methods: Pakistan, like most Asian countries has a predominantly bilingual fabric. Little children, regardless of their socioeconomic status are most often exposed to their native language (L1) and to a second language (L2) within the critical period (0–3 years), leading to simultaneous bilingualism. When they are exposed to L2 after the acquisition of L1 we call it sequential bilingualism. Some children may have L3 in their linguistic repertoire which may be the additional language such as the language of instruction at school. The development of articulation and phonological skills in children usually reaches maturation around the chronological age of 6 years. Children pick up several sounds of their language and perfect them gradually using approximations that sound closer and closer to the target sound, acquiring all sounds with the culmination of early childhood.

Results: Children may present with unclear speech, in the absence of an organic etiology (dyslalia or phonological disorders) or because of a known organic cause such as cleft lip/palate, malocclusions, hearing loss, dyspraxia or dysarthria, must be assessed for their ability to acquire phonemes of all the languages in their repertoire, because the brain stores auditory images of all sounds and uses them appropriately when needed.

Conclusions: The Tri-position Articulation Analysis for Bilinguals is a tool for testing the articulation and phonological skills of bilingual children aged 0–6 years, that uses culturally and linguistically appropriate picture stimuli for children exposed to Urdu, Sindhi, Punjabi, Pushtu, Hindi and English. The test assesses spontaneous elicitation of the sounds in the child's lexicon, along with stimulability and also assesses the function of the speech mechanism for non-speech functions.

Stability studies of metformin hydrochloride, Amlodipine besylate and Ciprofloxacin hydrochloride tablets under specified condition of temperature and relative humidity of Pakistan

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A pharmaceutical formulation ensures safety, protection, procurement and preservation of human health. Therefore, preservation of its physical and chemical stability throughout the period of its shelf life has always been the matter of prime concern for a pharmaceutical manufacturer. A good formulation is one that when packaged in a suitable container, (which provides maximum protection to its contents), confirms the absolute delivery of its active ingredient in its original form to its consumer. To determine the suitability of blister strips for packaging of Metformin HCl, Amlodipine besylate and Ciprofloxacin hydrochloride tablets, we maintained a stability profile for the changes in physical appearance and loss of potency of these medicines for a period of 2 shelf life years. Tablets were subjected to the specified conditions of 30 °C and 65% RH for 24 months and samples were withdrawn at different time intervals. Different tests and assays were conducted on these samples showed that under these specified conditions the efficacy of these tablets in the blister strips retain for less than 2 shelf life years which is less than the expected shelf life of 3 years.

Retrospective analysis of maternal mortality at Ziauddin UNIVERSITY Hospitals, from 2012–2014

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Introduction and objective: To determine the frequency of maternal mortality and identify the causes with different age groups, parity and gestational ages in Ziauddin university hospitals in 4 campuses, from 2012 to 2014.

Methods: A descriptive study conducted at Gynecology & Obstetrics units of Ziauddin university hospitals with retrospective review of validated records of hospital registers in 3 years from January 2012 to December 2014. Convenience sampling used to access the records. A self-structured proforma designed to collect data on variables (age, parity, gestational age, cause of death, condition at the time of admission, duration of stay in hospital before death, cause of delay).

Results: During 3 year period from January 2012 to December 2014 there were 32 maternal deaths. Total number of births were 14219 and 14184 were live births. Still births were 33. Eclampsia (21.8%) and hemorrhage (21.8%) were the leading cause of maternal mortality followed by those with puerperal sepsis (12.5%) and others. MMR (maternal mortality ratio) was 226 per 100000 live births.

Conclusion: Maternal mortality was high with leading causes as hemorrhage, eclampsia, and sepsis in ages of 20–30 years and parity 1–4. Mostly deaths were due to delay in getting the women to the health facility.

Vitamin D deficiency in pregnant women and their new born

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Introduction and objectives: Vitamin D deficiency may affect the mother and its new born health. The aim is to detect the frequency of vitamin D deficiency in pregnant women and their newborn of Karachi at tertiary care hospitals, and correlating maternal vitamin D with cord blood.

Methods: This cross-sectional study was conducted on 50 women in labor presenting with a singleton term pregnancy at tertiary centers in Karachi. Data was recorded on a special proforma, maternal blood was taken before delivery and cord blood was taken at delivery. All blood samples were analyzed for 25-hydroxy vitamin D levels.

Results: The mean vitamin D levels were 24 ng/ml for the

mothers and 20 ng/ml for the newborns. Vitamin D sufficiency was noted in 11 (22%), insufficiency in 16 (32%), and deficiency in 23 (46%) of the 50 mothers whereas sufficiency and deficiency, were noted in 6 (12%) and 44 (88%) of the newborns respectively. There was a positive correlation between the vitamin D levels in maternal and cord blood ($r=0.03$; $P=0.003$). Maternal vitamin D levels were significantly affected by sunlight exposure ($P=0.007$) and quality of diet ($P=0.01$).

Conclusion: Vitamin D deficiency is high among pregnant urban Pakistani women and their newborns. This public health problem needs urgent attention.

Are you prepared to care your loved ones: A cross sectional study to assess the level of preparedness among caregivers of chronically ill patients in a tertiary care hospital at Karachi, Pakistan

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Introduction: Family caregivers are also known as informal caregiver. They provide all level of care at home to their loved ones most often equivalent to the professional care providers, such as providing physical care, giving medications recognizing early signs of severe conditions, changing dressings etc. While providing care to their loved ones most of them feel unprepared in terms of lack of knowledge and skills. This unpreparedness may result in the role strain and have many psychological consequences for the family. Nurses as a primary care provider could assess the caregiver preparedness and may educate them according to the patient's need to reduce the extra burden and the role strain.

Method: The self-administered instrument regarding the preparedness for care giving scale by Archbold (1990) has been utilized. The scale has eight items to identify the preparedness of caregiver in multiple domains. The responses are rated on five point scale with scores ranges from 0 (not at all prepared) to 4 (very well prepared). The higher score means caregiver feels more preparedness and vice versa. 50 caregivers of chronically ill patients were selected at a tertiary care hospital, which were caring for their loved ones for about six months or above. Written consent was obtained from the participants.

Result: 50 caregivers were recruited to fill the form from which 26 were female and 24 were male. All of the participants were between 18 years and 60 years of age. About 68% caregivers score between not too well prepared and somewhat well prepared. Whereas, only 32% score between somewhat prepare and pretty well prepared.

Conclusion: In conclusion, most of the caregivers score are somewhat prepared which is an alarm for nurses and all health care providers to work to reduce their role strain and provide sessions according to the need of the caregivers. Nurses could arrange teaching sessions which could be helpful for the caregivers to do their role effectively.

ABO and Rhesus blood group distribution in residents of Karachi

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Introduction and objective: Blood is an essential component for human beings and in disease states, it is requested to be transfused from healthy human donors. But it was continuously a life threatening procedure until the discovery of ABO blood groups. Today committee of League of Nations has recommended A, B, AB & O classification for general use. This study was aimed to determine the frequency of ABO and Rh blood groups. So that necessary measures should be taken to maintain the blood product inventory. Frequency of ABO and Rhesus blood group distribution in residents of Karachi.

Methods: A study was conducted at Dr. Ziauddin Hospital

Clifton laboratory Karachi, Pakistan over a period of 7 months from 1st June until 31st Dec 2014. Healthy blood donors and patients requiring blood products of different age groups were employed. After an informed consent blood grouping was performed by taking blood samples under aseptic measures in EDTA and Gel separating serum tubes for ABO & Rh blood grouping by tube method.

Results: Total 1583 individuals included 868 (54.8%) males and 715 (45.2%) females. O+ve blood group found to be more common group (31.9%) followed by B+ve (31.2%). In Rh system, Rh+ve (97.2%) were more common than Rh-negative (2.8%) blood groups.

Conclusion: This study was taken out to maintain the blood product inventory concerning the management of blood bank and transfusion services for the patient.

Frequency of ABO blood group discrepancies in tertiary care hospital Karachi and their classification into groups

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Introduction: In developing countries, the blood grouping is generally being performed by the forward grouping method only. The aim of this study was to find out the frequency of ABO blood group discrepancies in tertiary care hospital in metropolitan city of Pakistan and also to classify these discrepancies on the etiological basis.

Methods: Cross-sectional, descriptive type of study was conducted at Blood Bank of Dr. Ziauddin Hospital Karachi, Pakistan. Total 1522 samples were included with exclusion of newborns and infants up to the age of 6 months. Blood samples were collected from a peripheral vein by trained phlebotomist and submitted to the blood bank department in 2 tubes, purple top vacutainer tube containing Di-potassium Ethylene Diamine Tetra Acetic Acid (K2 EDTA) anticoagulant and red top vacutainer tube containing clotted blood. Blood group testing was performed by tube technique.

Results: In total 1557 patients (854 Males and 703 Females) from various clinical specialties were included for ABO blood grouping. The male to female ratio was 1.2:1 (854 males and 703 females) with age ranging from 9 months to 92 Years. ABO discrepancies were Positive in 18 out of 1557 Patients (1.1%). Male to female ratio among ABO discrepancies were 1.5:1. There were four groups of ABO discrepancies. The most common group of discrepancies were group I discrepancies consisting of 12 out of 18 Patients (66.7%). Group II discrepancies were consist of 2 out of 18 Patients (11.1%). Group III discrepancies were consisting of 1 out of 18 Patients (5.5%) and Group IV discrepancies were consisting of 3 out of 18 Patients (16.7%).

Conclusion: The forward and reverse grouping are the essential parts of blood group testing for assigning the correct ABO blood group to the individual. ABO discrepancies i.e. forward and reverse grouping are not matches to each other can cause severe transfusion reactions which can be fatal for life. Furthermore, to ensure that correct ABO blood product to be transfused for saving life. Therefore reverse grouping should be strictly adopted in every blood bank and transfusion services as a routine practice.

Selfmedication practice among undergraduate medical students

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Introduction and objective: Assessment of self-medication practice among medical students is presumed to be of exceptional importance as in future they have promising role in counseling the patients regarding the rational use of drugs. This study was specially designed to evaluate self-medication practice in 3rd year medical students.

Methods: A cross sectional institution based study was

conducted from Jan – March 2013 on 3rd year medical students at Bahria University Medical and Dental College, Karachi, after approval by ERB-BUMDC. Data was collected by questionnaire, filled on one to one basis after taking verbal consent and analyzed on SPSS version 16.

Results: Frequency of self-medication was found to be 90%. Quick relief (38%) and busy schedule (21%) were the main reasons provoking self-medication. Common ailments that led to self-medication were: headache (95%) and fever (87%). Drug groups frequently used were: analgesics (95%), antipyretics (87%) and antibiotics (36%). Acetaminophen was the most common analgesic (73%) as well as the antipyretic (88%) while antibiotics were, metro-nidazole (26%) followed by amoxicillin plus clavulanic acid (17.3%).

Conclusion: Practice of self-medication is high among medical students. Potential problems of self-medication should be emphasized to the students.

Determination of prostate gland volume by ultrasonography and its correlation with anthropometric measurements in a subset of Karachi population

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Introduction and Objectives: To establish the local reference range of prostate volume according to our subset of population. To correlate prostate volume (PV) with age, body mass index (BMI) and waist circumference (WC).

Methods: A cross-sectional study with 119 healthy adults aged 40–79 years without any prostatic pathology were recruited. The study population was categorized into 4 age groups (40 - < 50 yrs, 50 - < 60 yrs, 60 - < 70 yrs, 70 - < 80 yrs), 3 BMI groups (healthy, obese and overweight) and 2 WC groups (< 90 cm and > 90 cm). A p-value of < 0.05 was considered significant. A total of 130 healthy adult volunteers were recruited initially, out of these 20 individuals were those whose IPSS was < 8 and prostate volume on ultrasound was > 25 ml. These 20 subjects underwent Uroflowmetry (UFM). After UFM, total 19 subjects were labeled as healthy adults and 1 subject was excluded from healthy adult group. The sample of 119 healthy subjects were studied sonographically for the effects of Age, BMI and WC on prostate volume.

Results: The mean prostate volume was 21.7 ± 2.2 mls, mean body mass index was 28 ± 6 kg/m², whereas mean waist circumference was 95 cm. PV was found to be higher in obese and > 90 cm waist circumference group. After applying multiple regression analysis, waist circumference correlated positively and significantly with prostate volume. UFM showed negative correlation with IPSS and PV.

Conclusions: Mean prostate volume in our studied population was smaller than that of many western populations. Our study has proved that central obesity is the most important factor influencing prostate volume.

Association of Kisspeptin, endometrial receptivity and unexplained infertility

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Introduction and objectives: To relate serum Kisspeptin levels with pregnancy outcome after intra cytoplasmic sperm injection (ICSI).

Methods: In the cross sectional survey carried at Australian Concept Infertility Medical Center from June 2014 to June 2015, 176 females with 20–42 years of age and with regular menstrual cycles were included for ICSI. Patients with uterine fibroids and metabolic disorders were excluded. Down regulation of ovaries was followed by controlled ovarian stimulation, ovulation induction, oocyte retrieval, microinjection and embryo transfer. Serum

samples for estimation of Kisspeptin, was done on OI day and endometrial thickness was measured. Based on beta hCG results were categorized into group A, non-pregnant with beta hCG < 25 mIU/ml, and group B, clinical pregnancy with beta hCG > 5 mIU/ml.

Results: Kisspeptin levels were significantly higher in the Group B versus Group A ($p < 0.001$) independently associated with positive pregnancy ($r = 0.388$; $p < 0.001$), and endometrial thickness ($r = 0.294$; $p = 0.05$) irrespective of the age and BMI of the subjects.

Conclusion: Kisspeptin is a positive pregnancy marker in females after ICSI as a result of its effects on oocyte maturation and endometrial thickness.

Effect of long hour shifts on medical interns and residents, and the strategies adopted to cope with work-related stress

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Introduction and objectives: To assess the impact of long hour shifts on performance and health of medical interns and residents and strategies adopted to cope with stress.

Method: This cross-sectional study was conducted from January to July 2015. A sample of 200 interns and residents was taken from Ziauddin Hospital Clifton, Kemari and North Nazimabad. Data was collected through a self-designed questionnaire and stress levels assessed through General Health questionnaire (GHQ-12). Data was analyzed through SPSS v 20. Chi-square and Paired T test was applied.

Results: The mean age of participants was 27 ± 2.3 years. Average sleep hours when doing call were 3.06 ± 1.47 . Work performance was below average in 62% of the population. Stress was found in 34% of study population. Increased tea intake was the stress coping strategy adopted by 72%. There was a significant association between stress level, gender and personal habits. ($p \leq 0.05$). A significant difference was found between cigarette and energy drink intake before and after starting job ($p \leq 0.05$).

Conclusion: High stress may have negative effects on health and performance. Hence, medical interns and residents need support to cope with stress.

Recurrent miscarriage and associated factors

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The incidence of recurrent pregnancy loss in women of reproductive age group is 0.5–3%, and idiopathic causes accounts for 50–60% of recurrent pregnancy losses. Approximately 30–50% of conceptions end in spontaneous miscarriage before the completion of first trimester. Miscarriages mostly occur at the time of implantation. The causes of recurrent pregnancy loss are many including parental chromosomal abnormalities, maternal thrombophilias, immunologic causes and endocrine disorders. Recurrent pregnancy loss is an extremely distressing clinical problem for women as well as health professionals. According to current studies the decidualized endometrium act as a biosensor of good quality embryos, if it is faulty, may lead to implantation of embryos resulting in miscarriage. Other factors implicated in the pathophysiology of miscarriage are Systemic and placental oxidative stress. Vascular endothelial damage, abnormal vascularity of placenta and immunologic reactions have been proposed to play some role in causing recurrent miscarriage.

The objective of this review is to discuss the causes of recurrent miscarriage, based on the published research articles.

Prevalence and preventive measures of prostate cancer: A randomized study in public and private health care sector

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Introduction and objectives: Prostate cancer accounts for

abnormal or uncontrolled division of cells of prostate in male. It is a slow growing cancer which is usually localized rarely metastasized. The growth rate varies from slow moderate to high. It mostly occurs in old age and depends on many other factors like weight, height, diet, habit, environment, genetics, and sexual disease. This study aims to evaluate occurrence, causes, problems associated with disease and treatment of this cancer in view of the fact to evaluate consequences responsible to contribute carcinoma of prostate.

Methods: For this purpose randomized study conducted at public and private health care sector (n=100 patients of prostate cancer).

Results: The Gleason score are used to diagnose prostate cancer, 10% of patients have score 5 (4+1), 40% of patients have score 7 (4+3) or (3+4), 44% of patients have score 9 (5+4), 6% of patients have score 11 (5+6) which shows that mostly prognostic grade II, III IV, V occur. Mostly traditional prostatectomy is used as a treatment and 78% of the patients got benefit from it.

Conclusions: This study concludes that the prostate cancer may occur due to age factor (old age), fatty diet, tobacco or alcohol intake. Finding of cancer at score 5,7,9,11 and associated complications are hypertension, urinary tract infection, kidney stones.

Knowledge and attitude towards menopause in women aged 45–60 years

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Introduction and objectives: Menopause has significant physical, mental, social and psychological effects on women's life and prior knowledge about these changes help them to cope with these changes with greater readiness. Educating women regarding menopausal changes would definitely equip them better to tolerate these menopausal symptoms.

Methods: A cross sectional study based on sample of convenience was conducted at the outpatient department of Ziauddin Hospital, Karachi from 1st July 2014 to 31st January 2015. The study participants were 250 menopausal women between the ages of 45–60 years. Data was collected by means of a questionnaire, which consists of three parts containing questions about demographic information, knowledge and attitude.

Results: The mean age of respondents in the current study was 51.9 ± 4.2 years. The mean age at menopause was 45.9 ± 3.8 years. As far as the knowledge about menopause is concerned 4.8% had weak, 71.2% had moderate and 24% had good knowledge about menopause phenomenon. Majority of respondents had positive (72.4%) attitude towards menopause and 27.6% had negative attitude towards menopause.

Conclusions: Appropriate knowledge and positive perception towards menopause is very important to help women to cope with menopause transition and can help in the development of appropriate programmes to promote women health.

Social support as a determinant of internalized stigma and self-esteem in persons with schizophrenia

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Introduction and objectives: Social support works as a buffering factor in psychotherapeutic treatment for the diagnosed persons with schizophrenia in reducing internalized stigma and in boosting their self-esteem. The aim of the present study is to explore the predictive relationship of social support with internalized stigma and self-esteem among diagnosed persons with schizophrenia.

Methods: A purposive sample of 52 diagnosed persons with schizophrenia, age ranges 18–55 years who belong to different socioeconomic status was taken from different psychiatric hospitals of Karachi. Demographic Information sheet, Multi-

Dimensional Scale of Perceived Social Support, Internalized Stigma of Mental Illness Scale (ISMI) and Rosenberg Self Esteem Scale were administered individually.

Results: Findings revealed social support predicts internalized stigma and self-esteem in persons with schizophrenia ($p < 0.000$).

Conclusions: There is an immense need to develop awareness regarding social support group and to generate strategies to reduce the effect of internalized stigma in person with severe mental illness.

Efficacy and safety of Metformin in gestational diabetes mellitus

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Objective: To assess the efficacy and safety of Metformin for the management of gestational diabetes Mellitus (24 wks and above) as compared to Diet.

Methods: This Quasi experimental study is being conducted on 600 females at Ziauddin University Hospital. All pregnant women receiving prenatal care fulfilling the inclusive criteria will be screened using a 50 g glucose challenge test (GCT). After the result if it is positive, women will be advised for oral glucose tolerance test. After diagnosis of gestational diabetes Mellitus patients will be advised for diet control or oral hypoglycemic drug metformin starting from 500 mg twice a day. Patient will be followed fortnightly with sugar record for adjustment of treatment. Patient will be followed till delivery for any maternal or perinatal complications. Patients with systemic diseases are excluded from the study.

Results: Result of this study are awaited as this is an ongoing study.

Conclusions: The main purpose of the treatment is to prevent fetal hyperinsulinemia and fetal macrosomia by reducing maternal glucose level, decrease risk of appearance of diabetes in subsequent pregnancies and in future without pregnancy. Metformin is an alternative to insulin and is effective in the treatment of gestational diabetes.

Impact of pharmacist intervention on antibiotic prescription orders in pediatric setting

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Introduction and objective: The prospective study was designed to formulate and test the efficacy and impact of trial interventions by pharmacist on potential harm and incidence rate of errant antibiotic medications dispensed in children admitted to a public sector hospital in Karachi.

Methods: An eight months study was conducted in a teaching hospital comprising of 850 beds with 65 beds strength in three pediatric units I, II and III inclusive of neonatal ICU. Orders written by physician groups in all patient admission categories were included. The prescription order review prior to dispensing was made by experienced and specialized pharmacists in pediatric pharmacotherapy in a research setting. Prescription order in error was marked if not in compliance with standard pediatric references, dosing guidelines and antibiotic prescription guide adopted in the hospital. As per predefined criterion in our study, errors were labeled as potentially lethal, serious and significant. The data was statistically compared by unpaired samples test and the efficacy of the intervention on type of errors was margined by chi square test.

Results: 8.5 medication errors per 1000 prescription orders were identified (1.5 medication error per 50 patient per day), where-as 18 potentially lethal errors were identified within the span of 8 months. Potential cardiopulmonary arrest and anaphylactic risk was significantly assessed ($p < 0.01$) in ICU. Overdose and under dose of antibiotics accounted to 76% errors. The

frequency of errors was comparatively non significant in prescription orders by senior attendees ($p < 0.05$).

Conclusions: Effective control of antibiotic medication errors is observed with the integrated role of pharmacist in the prescription-dispensing-administration sequence.

Simultaneous determination of six antihistamine anti-allergic drugs in pharmaceutical formulations, human serum and pharmacokinetics application

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This article describes a new, accurate and highly specific high performance liquid chromatographic method with UV detection (HPLC-UV) for the simultaneous determination of cetirizine HCl, chlorphenamine maleate, loratadine, domperidone, buclizine and meclizine in pharmaceutical dosage form and human serum, involving pyridoxine as internal standard. The mobile phase consists of heptane sulphonic acid salt buffer and acetonitrile, drawn at a flow rate of 1.0 ml/min using a symmetry C18 column with UV detection at 230 nm. The intraday and inter-day precision measurements showed coefficients of variation always less than one. The calibration curve was tested in the range of 10–2150 ng/ml and the correlation coefficient of > 0.9990 in all cases was obtained. The averages of the absolute and relative recoveries were found to be in the range of 98–102%. Up to six drugs were separated in the same chromatogram with good resolution. The proposed HPLC method has reasonable applications in pharmaceutical tablet dosage form and pharmacokinetics studies.

Simulation based cardiopulmonary auscultation: a tool to aid medical students of Ziauddin University

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Introduction and objectives: Use of simulation and state of art mannequin and models in clinical skills lab is now becoming a common practice which is offered to 3rd year medical students during their clinical rotations. We seek to determine the efficacy of cardiopulmonary auscultatory skills in 3rd year medical students of Ziauddin University who have been trained by simulation based auscultatory device.

Methods: Students who participated in this study ($n=40$) were from 3rd year MBBS program. They were assigned in 2 equal groups. Group A took a lecture on the detection and knowledge of various cardiopulmonary sounds whereas Group B was tutored on a simulation based device after they had taken their lecture. After respective training sessions, each group was tested with the help of a questionnaire. This questionnaire consisted of identification and knowledge based open ended and close ended questions. Data was entered into data analysis software S.P.S.S ver 15, Data was analyzed and mean, standard deviation was calculated along with Charts, graphs were generated to represent results.

Results: Diagnostic accuracy and correct identification of cardiopulmonary sounds was better in the group that had been assisted with a simulation based device. Mean score of assessment of knowledge and skills of cardiopulmonary sounds were found significant in group B (mean = 13.5 ± 1.73) compared to group A (mean = 6.9 ± 2.64).

Conclusions: According to our study at Ziauddin medical university, it is suggested that simulation based learning is a subsidiary tool in preserving knowledge and skills for cardiopulmonary auscultation.

Whole fruit better than fruit juices in maintaining blood sugar levels

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Introduction and objectives: Type 2 diabetes mellitus (T2DM), non-insulin dependent diabetes mellitus, is the most rapidly growing disease, estimated to affect 439million adults by 2030. The rate is faster in developing countries than developed countries which in turn results in higher healthcare expenditure. Thus it becomes a public health priority to prevent the disease and control over its rapid growth. Among the known risk factors that contribute to T2DM are dietary factors, which promote early diabetes in healthy individuals and increased mortality and morbidity in type 2 diabetic patients. To evaluate the hypothesis that fruits provide more protection against T2DM than fruit juice, we performed experiment by giving fruits to one group and fruit juice to another group of healthy volunteers and monitor fasting blood sugar and random blood sugar.

Results: The results of current study suggested that whole grapefruit and orange fruit are very effective in maintaining random blood sugar levels in comparison to juices.

Results also suggested that dietary fibers are not only effective in regulating blood sugar, but also showed insignificant change in systolic and diastolic blood pressure and also in pulse rate. Moreover, whole grapefruit has been found more effective in maintaining pulse rate in comparison to fibers obtained from apple fruit and orange fruit.

Conclusions: Based on above findings, it has been concluded that patients with type 2 DM could be maintained on dietary fibers not only for better glycemic control, but also for controlling diabetes related complication such as high blood pressure. This study could further be extended to assess the effects of dietary fibers (especially obtained from grapefruit) in controlling other diabetes related complications, e.g. hypercholesterolemia, diabetic nephropathy, in diabetic patients.

NAFLD in diabetic patients treated on oral hypoglycemic

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Introduction and objectives: Determination of NAFLD (non-alcoholic fatty liver disease) in diabetic patients on oral hypoglycemic therapy. Diabetes is one of the most common non-communicable disorder throughout the world. It has enormous complications, which indirectly multiply the economic and medical burden. According to WHO, 9% population of the world suffers from Diabetes who are above 18 years age. Prevalence of diabetes in Pakistan is about 10% according to Akhtar. This poses a huge burden on a country like Pakistan, which already has very limited resources in field of health.

Deficiency of Insulin causes hyperglycemia and hyperlipidemia. This leads to multiple disorders like atherosclerosis, CVAs, IHD, Renal failure, NAFLD, glaucoma, cataracts, gangrenes of limbs, acute emergencies like DKA (diabetic ketoacidosis) and HONKC (hyperosmolar non ketotic coma) etc. NAFLD (Non Alcoholic Fatty Liver Disease) was first described by Ludwig in 1980 and is on rise, making it the most common cause of CLD. Commonly called fatty liver diseases is a major problem arising in diabetic population, which has been neglected off until now. The prevalence rises to 60.8% in diabetics in Pakistan. 40% cases of NAFLD can progress to NASH (nonalcoholic steatohepatitis) of whom 32–37% can progress to advanced fibrosis. This prevalence rises to 35–75% in obese and diabetics. Making it most common cause of cryptogenic cirrhosis, which can lead to hepatocellular carcinoma. NAFLD is usually an incidental finding when an abdominal scan is done for some other suspected pathology.

Methods: FBS, Fasting LIPID profile and HbA1C was done. Ultrasound was done to see fatty infiltration of liver.

Conclusion: Ultrasound liver of type II diabetic patients showing non-alcohol fatty liver changes.

Identifying errors in prescription writing

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Introduction and objective: The errors of prescribing are the commonest form of avoidable medication errors and are considered to be the most important target for improvement. Medication errors are common in general practice. Both errors in the act of writing (prescription errors) and prescribing faults due to erroneous medical decisions can result in harm to patients. Our objective is to identify the errors in the prescriptions of general practitioners (GPx) from different parts of Karachi.

Methods: A descriptive pilot study was conducted from 1st January to 30th February 2014. A total of 100 prescriptions were collected from 4 general practitioners' clinics (east, west, central and south districts) of Karachi. Verbal consent was taken few days prior to random collection. All prescriptions were analyzed for errors in superscription, inscription, subscription, transcription, signatures and refill information.

Results: A total of 373 errors were identified in 100 prescriptions. 203 errors in superscription, 6 in subscription, 34 in transcription, 1 in prescribers' signatures and 62 in refill information.

Conclusion: Errors in prescription writing are found to be common in the prescriptions of general practitioners. Measures should be taken to refresh the prescription writing skills of general practitioners through CMEs and workshops.

Assessment of stressors among undergraduate dental students of Karachi, Pakistan

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Introduction and objectives: Dental schools are known to have highly stressful learning environments. Dentistry involves an acquisition of required academic, clinical and interpersonal skills during the course of learning. Identifying sources of stress represents the crucial first step towards advocating policy changes and enhance student's stress coping skills. This study was conducted to assess the problems related to stress encountered by dental students of Karachi. The aims are: (a) To identify the common stressors among undergraduate dental students of Karachi. (b) To investigate whether specific stressor is related to year of study or gender. (c) To evaluate the factors which influence student perception that might allow programmatic changes.

Methods: Type of Study: Cross Sectional Survey; Sample Size: 330 undergraduate dental students of Karachi; Study population: second, third and final year BDS; Study setting: Five dental schools of Karachi; Modified form of Dental Environment Stress questionnaire was administered.

Results: A total of 500 Questionnaire were distributed out of which only 330 responded. Of the respondent, 70% were women and 30% were men. The most stressful variable among all was 'Overloaded feeling due to vast syllabus'. Third and final year students were reported to have higher-level stress than second year students. Females were more affected by the same stressor than male students.

Conclusions: The conclusion of study was drawn from variables with significant values < 0.005 . Stress due to accommodation problem was higher in students of second year while clinical based problem caused greater level of stress among students of third and final year. The variables relating to academics (inadequate break timings, inadequate time for assigned work and overloaded due to vast syllabus) caused stress in all three years equally. Stress showed an upward trend from the first to the final year with a jump from the second to the third years.

Frequency of depression in house wives and associated risk

factors

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Introduction and objectives: In any population, women are more prone to depression. In our middle class community it is still considered a taboo. Housewives usually emphasize on somatic issues rather than emotional signs and symptoms which causes detrimental effects on their health. Timely identification of risk factors and counseling of women regarding their mental health is of utmost importance. We aim to measure frequency and identify major risk factors of depression in housewives belonging to middle class community in Karachi.

Methods: Cross sectional study conducted on housewives between 25 and 50 years of age. Through convenience sampling technique 247 questionnaires with 17 questions were administered, followed by the patient's health questionnaire 9 (PHQ-9). Data was analyzed by SPSS v20. Chi-test was calculated for categorical data and p-value < 0.05 was considered significant.

Results: Out of all the respondents, 131 females (53%) turned out to be depressed and 116 (57%) not depressed. PHQ-9 classification of the levels of depression in the participants showed 100 females mildly depressed, 77 females moderately depressed, 35 females moderately-severely depressed, 16 females severely depressed and 19 females not depressed.

Conclusions: Most prominent risk factors for depression were of having to take care of an old or an ill person at home and living in a joint family structure. Although the study targeted middle class community, the analysis showed no associations with the family income.

Frequency and type of chewable tobacco commonly used by oral squamous cell carcinoma patients

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Introduction: South East Asia is threatened by the risk of Oral Squamous Cell Carcinoma. The popularity of chewable tobacco plays a pivotal role in making it the second leading malignancy in Pakistan. Prolonged use of these products can lead to different oral precancerous conditions, GI cancer, ulceration and many other diseases. The objective of the study was to find out the frequency and type of chewable tobacco, which has been the cause of Oral Squamous Cell Carcinoma in these patients.

Methods: The study was descriptive. Seventy one patients who had Oral Squamous Cell Carcinoma and a history of tobacco use were selected from cancer OPD. Subjects addicted to stuff other than smoking and chewable tobacco were excluded. A questionnaire was used to collect demographic characteristics, oral hygiene, medical history, family history of cancer, and detailed history of tobacco usage, chewable or smoke products and details of chewing habits (using since, packs/day, duration of exposure etc.). The study was carried out in Ziauddin.

Results: Majority of the subjects were males (80%). Out of the 71 Oral Squamous Cell Carcinoma patients majority 42 (59.15%) were multiple users that consumed more than one tobacco product. Whereas, 9 (12.67%) consumed gutka, 9 (12.67%) pan, 5 (7%) betel nut, 4 (5.6%) niswar and 2 (2.8%) cigarette.

Conclusions: Frequency of OSCC was found higher in users of multiple chewable tobacco formulations which makes consumption of multiple tobacco formulations one of the major etiological factors in the development of OSCC.

Molecular journey of Vitamin D, VDR and VDRGP and its association with breast cancer

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Vitamin D plays an essential role in regulating the levels of

calcium and phosphate in the body. Vitamin D is not active as a hormone - instead, a few chemical changes must be made. These are performed by enzymes that add hydroxyl groups. Vitamin D receptor is a nuclear hormone receptor Transcriptional regulator in response to 1,25, dihydroxyvita-min D 3.

VDR protein is at the center of the Vitamin D endocrine system with a negative feedback which regulates serum calcium and Vitamin D, if not maintained will affect large number of organs including breast. Data is available in positive genetic association of certain diseases which suggest functional consequences of VDR gene polymorphism. Vitamin D plays a role in breast cancer and exerts its effect through VDR gene polymorphism. There are four polymorphic variants of VDR (Folk 1, Bsm1, Taq 1 and Appa1). Hormone Receptor-Vitamin D hormone binds to receptors in its target cells, controlling the synthesis of many different proteins involved in calcium transport and utilization. The receptor is composed of two domains: a domain that binds to the hormone and a domain that binds to DNA. It pairs up with a similar protein, 9-cis retinoic acid receptor (RXR), and together they bind to the DNA, activating synthesis in some cases and repressing it in others. There is a decrease in VDR expression in breast cancer cells compared to normal breast cells and this decrease could be due to gene polymorphism and other factors also Alterations in VDR expression and activity could lead degranulation of vitamin D uptake, metabolism and serum levels of biologically active Vitamin D.

Potential of black mulberry: From food of choice to remedy for bowel dysmotility

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Introduction and objectives: *Morus nigra* Linn. (black mulberry) is used in gastrointestinal ailments. This study demonstrates gut modulatory properties of *M. nigra*.

Methods: The prokinetic, laxative and antidiarrheal activities of *M. nigra* were assessed in mice, while isolated rabbit jejunum and guinea-pig ileum were used to explore insight into mechanism(s).

Results: At 30 and 70 mg/kg, the crude extract of *M. nigra* (Mn. Cr) exhibited atropine-sensitive prokinetic and laxative effects, similar to carbachol (CCh). While at higher doses (100–500 mg/kg), Mn. Cr offered protection against castor oil-induced diarrhea.

In rabbit jejunum, Mn. Cr and its chloroform fraction inhibited CCh-induced contractions more potently compared to high K⁺ (80 mM). Conversely, petroleum fraction was more potent against

high K⁺-induced contractions. At 0.01 mg/ml, Mn. Cr caused a parallel shift in acetylcholine (ACh) concentration-response curves (CRCs) followed by a non-parallel shift at 0.03 mg/ml, similar to dicyclomine. At further tested concentrations, Mn. Cr (0.1 and 0.3 mg/ml) and petroleum fraction suppressed Ca²⁺ CRCs, similar to verapamil. In guinea-pig ileum, Mn. Cr, its aqueous and ethyl acetate fractions exhibited atropine-sensitive gut stimulant activity along with additional uncharacterized excitatory response in aqueous fraction only.

Conclusions: These results suggest that black mulberry possesses prokinetic, laxative and antidiarrheal effects, putatively mediated through cholinomimetic and anti-muscarinic plus Ca²⁺-antagonist mechanisms, respectively.

Antibacterial activity of honey and turmeric against git and respiratory pathogens

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Introduction and objectives: Because of emergence of resistant pathogens the antibiotic activity is diminished. This poses a serious threat to public health. Therefore alternative antimicrobial techniques are needed. Thus this situation has led to reevaluation of the therapeutic use of ancient remedies such as plants and plant based products including honey and turmeric.

Methods: The study was performed to evaluate the antimicrobial activity of honey and turmeric, purchased from local market. Different concentrations of honey and turmeric were prepared in distilled water including 100%, 75% and 50%. Its effect was observed on isolates obtained from clinical samples, which includes *Salmonella typhi*, *Shigella dysentrey*, *S.aureus*, *Proteus mirabilis*, *E.coli*, *Pseudomonas aerignosa*, *Klebsiella oxytoca*, *Bacillus subtilis*, *Enterococcus* and *Micrococcus leutus*. The study was carried by disc diffusion method. All experiments were performed in triplicate. Zone of inhibition was measured in millimeter and results were given in percentage.

Results: Honey extract show highest antibacterial activity as compared to turmeric extract. Highest concentration (100%) of honey and turmeric was inhibitory to all strains among which *Enterococcus* gave 75% inhibition while other strains were inhibited up to 50%. It was found that 75% concentration of these compounds gave intermediate zone of inhibition while 50% concentration was least inhibitory.

Conclusions: Honey and turmeric have potential to use as therapeutic agents either alone or in combination against various infections.

Abstracts: Joint 2nd Annual Conference of Ziauddin University, HEC Pakistan with European Society for Translational Medicine (EUSTM) International Conference on Translational Medicine: From Discovery to Health Care

Oral Presentations

DR. N.A. JAFAREY MEMORIAL LECTURE

CO-ADMINISTRATION OF TURMERIC AND KALONGI (BLACK SEEDS) SHOWED ENHANCED EFFICACY IN METABOLIC DISORDERS – FROM BENCH TO BED, TRANSLATIONAL STUDY

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Amongst non-communicable diseases, metabolic syndrome (MS), a cluster of metabolic disorders including obesity, hyperglycemia, dyslipidemia and hypertension, is highly prevalent in modern society. Due to its rising incidence and demanding life-long use of multiple drugs, there is a growing interest in testing and developing safe alternative remedies.

Animal studies were conducted to establish a better rat model of MS with low HDL. Replacing whole wheat with refined wheat flour in rat chow in 60 % fructose-fed Sprague-Dawley rats resulted in hypertension, hyperinsulinemia, hyperglycemia and a reduction in HDL levels at 4 weeks, while hypertriglyceridemia with endothelial dysfunction was observed at 8 weeks. This rat model was therefore, used to study the efficacy of co-administration of Turmeric and Kalongi on parameters of MS. The HPLC fingerprinting of the crude methanolic extract of Turmeric showed the presence of curcumin mainly with a few minor unidentified compounds while, the extract of Kalongi seeds showed the presence of thymoquinone along with some other unidentified compounds.

At 3 weeks of the treatment of rats with Kalongi (0.3 g/kg) and Turmeric (1.5 g/kg) lowered raised blood pressure and low density lipoprotein (LDL), respectively, while their co-administration at half the dose of individual herb reduced blood pressure and triglycerides. At 6 weeks, higher dose of Kalongi alone (0.6 g/kg) prevented hypertension, hyperglycemia, dyslipidemia and endothelial dysfunction but had no effect on cholesterol and serum insulin levels when compared with fructose-fed controls. Turmeric alone, in a higher dose (3 g/kg) prevented dyslipidemia, but had no effect on blood pressure. Co-administration of half the doses of individual herbs (Turmeric 1.5 g/kg and Kalongi 0.3 g/kg) offered better efficacy in wider range of parameters of MS such as prevention of hypertension, hyperglycemia, dyslipidemia, hyperinsulinemia and endothelial dysfunction.

Study conducted on human revealed that around 30 % were overweight with nearly 60 with central (abdominal) obesity and when Kalongi and Turmeric given in combination with 60% dose of the individual herbs, showed an improvement in all studied parameters of MS including in body fat, cholesterol (also HDL) and blood glucose. The results therefore, for the first time, revealed enhanced effect of a low dose combination of Turmeric and Kalongi on parameters of MS among animals as well as humans and this study has huge impact on public health offering relative-ly safe and cost-effective remedy for some chronic diseases.

This study was part of PhD thesis of Dr. Faridah Amin graduated from Aga Khan University in 2015.

UNDERSTANDING TRANSLATIONAL MEDICINE

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In the recent past, Translational Medicine (TM) has gained significant importance due to its promising role for accelerating research and development in academia and industry. TM has great potential to enhance health care and better patient care starting from prevention, diagnosis, and drug/device developments for clinical disorders. Translational medicine tools and techniques help to plan and improve infrastructures and capabilities while reducing cost and optimizing resources. This talk will provide an overview of translational medicine field including definitions and concepts, techniques and tools, potential benefits as well as global initiatives and programs.

DIGITAL PATHOLOGY – AUTOMATED RECOGNITION OF CANCER CELLS FROM BODY FLUIDS

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The Talk will cover the full spectrum of topics currently making an impact on the digital pathology practice. Whole slide imaging has the potential to improve efficiency and cost-effectiveness, while providing access to high quality care in areas that are currently under-served. Many institutions are leading the way with the use of whole slides images for primary diagnosis, while others are trailing behind. Progress being made in digitization and recognition and the advantages of taking strides to digitize information to share images and data across institutions as well as encourage adoption by medical institutions and thereby lending expertise across geographic borders will be evaluated. Technical progress in new technology, data analysis, machine learning and IT infrastructure in addition to standardization of practice will be highlighted with emphasis on non-invasive cancer diagnostics based on liquid biopsies.

PHYTOMEDICINE FOR HEALTHCARE IN THE 21ST CENTURY

M Iqbal Choudhary¹ and Atta-ur-Rahman¹

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Global healthcare system is challenged by emerging diseases, equity issues, and most importantly sustainability. Nature provided perfect system to nature, sustain and improve life for human and other life forms since the beginning. Some time during the last century, capacity to synthesize new molecules has made its way in drug discovery and development. The mutual co-existence of both systems, i.e. nature-based healthcare and synthetic based medicines, could have been much better, but obsession of pharmaceutical industries to quickly bring blockbuster drugs to market-place totally distorted the focus. Overemphasis on synthetic medicines has not only seen the world's indigenous knowledge and practices diminished, but also marginalized and disowned the indigenous healthcare systems which supported the human race for so long. Today there is a major disconnect between the two

systems, where nature-based drug discovery is grossly neglected. Cost of bringing a new drug is extremely high (US \$ ~ 2.0 billion), leaving poor man and orphan diseases untreated. Intellectually the developing world has no role in the global drug discovery and development process. Business of pharmaceutical development is not-able to meet the needs of majority of human population. Ironically interest in nature-based drugs has never diminished. More so inability of modern medicines to treat human diseases and their reported side effects created a niche for herbal-based drug discovery and development.

The phyto-medicine too despite their general acceptability, growing interest and promise, face great challenges, such as fast disappearing indigenous knowledge, loss of habitat of medicinal plants, proof safety and efficacy, and biopiracy. The presentation will be an analytical review of the importance of herbal and natural medicines in 21st century, along with some of the most enduring challenges this is facing. Unfortunately many of these challenges are not even discussed.

ADVANCED GLYCATION ENDPRODUCTS: POSSIBLE LINK BETWEEN DIABETES AND BREAST CANCER

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Diabetes mellitus affects over 382 million people worldwide and this number is expected to increase to 592 million by 2035. Diabetes is characterized by hyperglycaemia, a consequence of which is increased protein glycation and the subsequent formation of complex heterogeneous molecules called advanced glycation endproducts (AGEs). Diabetic patients often have an increased susceptibility to certain types of cancers including breast cancer. Although AGEs have been implicated in many diabetic complications, their effects on breast cancer cells are not well studied. This study investigates the effects of AGEs on the invasive MDA-MB-231 breast cancer cell line and showed that AGEs increased proliferation, migration and invasion of these cells in a dose-dependent manner. Furthermore, AGEs also up-regulated the expression of the receptor for AGEs (RAGE) and of the key signalling molecules. Blockage of AGE interaction with RAGE inhibited AGE-induced effects including signalling pathways in this breast cancer cell line. Therefore, AGEs might contribute to breast cancer development and progression in diabetes. Antiglycation compounds may have a role in treating diabetic patients with breast cancer.

USE OF MUSCLE SPLITTING BIPLANE IN PRIMARY AND REVISIONARY BREAST AUGMENTATION AND AUGMENTATION WITH MASTOPEXY.

Umar Daraz Khan¹

¹*Aesthetic Plastic Surgeon, United Kingdom.*

Introduction: In Muscle Splitting Biplane technique, muscle lies in front and behind the implant at the same time. Submuscular positioning of the implant is achieved by splitting muscle along its fibres direction without muscle release. The concept is used for augmentation mammoplasty and augmentation mastopexy in primary and secondary cases.

Methods: Since 2005, author performed 1670 implant related surgeries. The technique was used in primary augmentation mammoplasties (1298), primary augmentation mastopexies (108), multiplane pocket for augmentation and internal glandulopexy through infra mammary crease (63). Muscle splitting biplane is also performed in patients requesting for change of implants following partial submuscular and subglandular mammoplasties (75), patients presenting with synmastia (6), bottoming down (26), capsular contracture (30), rippling of the breast (30), rupture of implants (9) and revision mastopexies following subglandular implant position (13). Author has also used the pocket to correct dynamic deformity (12) seen following partial submuscular breast augmentation.

Results and Conclusion: Majority of the patients were happy with the results. Muscle splitting biplane is a versatile pocket and its quick learning curve and reproducibility has made it a good option for augmentation mammoplasty, augmentation with mastopexy in both primary as well revision procedures.

NUTRACEUTICALS: THE SCIENCE BEHIND THE MYTH

Khalid Rahman¹

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Epidemiological studies have shown that diets rich in plant foods can either reduce or prevent chronic diseases e.g. cardiovascular disease. Medicinal plants and nutraceuticals usage can be a cost effective way of sustaining and improving general health especially in countries with limited health resources. The concept of healthy ageing and personalised medicine is also becoming important. We have investigated the role of garlic in the prevention of cardio-vascular disease and the role of green tea in promoting neutrophil activity both in vitro and in vivo. Garlic has attracted considerable interest as a potential cardio-protective agent whilst green tea is reported to enhance the immune system. Garlic was given to healthy volunteers and it displayed antioxidant properties as judged by an increase in total antioxidant status and inhibition of LDL oxidation; further more garlic prevented platelet aggregation by mechanisms which include an increase in cyclic nucleotides, inhibition of fibrinogen binding and platelet shape change. Neutrophils play a defensive role against bacterial infections by oxygen dependent and independent pathways. Neutrophils were isolated from healthy volunteers and primed and activated with formyl peptide fMethionine-Leu-cine-Phenylalanine (fMet-Leu-Phe) and lactoferrin and myeloperoxidase was measured as well as water soluble antioxidant status. Green tea extract was then given to volunteers and the assays repeated; increase in lactoferrin, myeloperoxidase and water soluble antioxidants was observed. In conclusion, garlic has the potential to reduce cardio-vascular disease whilst green tea may protect against bacterial infections and both garlic and green tea have the potential to protect against oxidative stress in healthy volunteers. Nutraceuticals have an important role to play in the field of Translational Medicine.

TRANSORAL LASER APPLICATION FOR VOICE CONSERVATION AFTER SURGERY FOR EARLY GLOTTIC CANCERS

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Introduction: The surgical laser has significantly improved the ability of the head and neck surgeon to work within confined spaces. It permits meticulous removal of cancerous tissues within the larynx without damage to adjacent tissues.

All cancer of larynx patients were resected with CO2 Laser at Tata Memorial Hospital

The voice quality of these patients was assessed objectively to find out the improvement in it.

Voice parameters assessed were:

1. Frequency Parameters
2. Intensity Parameters
3. Perturbation Characteristics
4. Vocal tract Parameters

Results: Demography of Patients is as shown below: n = 2380
T1 Glottic Cancers All male patients

Median Age = 56 years (range 27 – 73 years) No adjuvant treatment was offered to them. Evaluation

All patients evaluated more than 3 months after treatment. Vowel /ee/ sustained for 5 secs.

Patients explained, trained and later evaluated Microphone to mouth distance of 3 inches Recording of speech and its analysis

using Dr.Speech Voice Evaluation Software ver. 5

SPSS ver. 10 was used for Statistical Analysis Method

Conclusion Voice after laser resection of the vocal cord is qualitatively affected.

Regular voice therapy is required to maintain the voice characteristics.

Patient needs to be educated in Vocal hygiene to avoid further damage to voice.

CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. It constitutes approximately 25% of cancers in children below 15 and 19% in children below 20 years of age. Several risk determining variables have been identified in childhood ALL and include patient-specific, clinical, and genetic features. Children treated on current chemotherapy protocols have an event-free survival (EFS) that exceeds 85%. This success is mainly due to treatment stratification according to the risk of relapse and provision of better supportive care. It is essential to know the disease characteristics and prognostic criteria to stratify treatment.

MUTATIONAL LANDSCAPE OF HEAD AND NECK SQUAMOUS CELL CARCINOMAS IN PAKISTANI POPULATION

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Introduction: Head and Neck Squamous Cell Carcinoma (HNSCC) is the most commonly diagnosed cancer in males and the second most common in females in Pakistan. The increasing burden of HNSCC in the region along with a unique set of risk factors merits a deeper investigation of the disease at the genomic level.

Methods: To this end, we performed whole exome sequencing of HNSCC treatment-naïve, HPV-negative cases and matched normal genomic DNA (n=7).

Results: We identified previously reported recurrent mutations in TP53. Novel recurrent mutations in HNSCC in the Pakistani population have been provisionally identified in several genes, including SERPINA3, CLMN, CHEK2 and PAK2. Using the IntOGen-Mutations platform, we identified cell cycle and MAP kinase related pathways among the most commonly affected pathways.

Conclusion: This data will be validated in a larger cohort and following functional analysis, the strengthened mechanistic knowledge could contribute new, rational therapeutic targets for the treatment of HNSCC.

LONG TERM NON PROGRESSORS: AN EXCELLENT MODEL TO STUDY NATURAL HIV CONTROLLING MECHANISM FOR THERAPEUTICS

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Infection with the immunodeficiency virus (HIV-1) leads to the development of the acquired immunodeficiency syndrome (AIDS) in humans that finally causes the death of the patient. To date, HIV lacks a suitable vaccine as almost all human HIV vaccine trials have invariably failed in vivo. Emphasis is now shifting to explore the natural mechanisms of HIV control that has been previously well detected in long term non-progressors. In contrast to normal progressors (AIDS patients), approximately 5-8% of all HIV infected people fall into the group of long-term non progressors (LTNPs) or Elite controllers (EC). These individual are infected with the virus for more than 10 -20 years but do not show clinical symptoms of AIDS. Thus they maintain a very low viral load by certain natural mechanisms that are still not fully understood. LTNPs serve as an ideal model for HIV-1 vaccine development due to their natural control of HIV-1 infection, since an understanding of their ability to control HIV infection may lead to the development of immune

therapies or a prophylactic/therapeutic vaccine. Previous studies have shown that viral, genetic and immunological components of these LTNPs play a role in containment of the virus. My presentation will focus on a few studies from own group as well as future projects with reference to HIV studies in Pakistan.

CURRENT STATUS OF TRANSLATIONAL NEUROSCIENCE RESEARCH IN PAKISTAN

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The enriched history of neuroscience research dates back to 4000 B.C., when euphoriant effects of poppy plant were reported in Sumerian records with the breakthrough of 52 discrete cortical areas by Korbinian Brodmann in the 19th century.

The potential impacts of advancements on science, medicine, industry and society are expected to be profound. Translational neuroscience is a relatively new term in brain research focusing on a closer interaction between basic and clinical neurosciences to expand understanding of brain structure, function and disease, and translate this knowledge into clinical applications and novel therapies of nervous system disorders.

Pakistan being a developing country is much behind in brain research as compared to its neighboring countries. There is a pressing need to establish a national body that encompasses all types of neurosciences including behavioral, cognitive, computational, molecular, neural aging, neuroanatomy, neurobiology, neurochemistry, neuroendocrinology, neuropathology, neuropharmacology, neurophysiology and systems neuroscience. There is also a need for a large neuroscience center in Pakistan which can train students in the neurosciences at the undergraduate and graduate level.

Unfortunately, in Pakistan as in most parts of the world, mental health and mental disorders are not accorded the same degree of importance as physical health. Rather, they are and have been largely ignored or neglected. One of our projects on translational neuroscience is based on revealing differential expression of nuclear proteins in Alzheimer's disease and Schizophrenic's human brain. We end up with discovering four major proteins to be differentially expressed in AD brain including histone H4, H2B1B, TUBB4B and VDAC1. Further studies are needed to confirm this aberration and develop novel therapeutic targets for AD.

MENOPAUSE BOON OR BANE

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Information regarding menopause is constantly evolving. Recent review of literature is being presented. Studies conducted in Ziauddin University showed majority of the young women are aware of osteoporosis, the most common long term health implication of menopause. It also showed that the main sources of awareness in Pakistan are doctors whereas in most countries the mass media and families are the main source. There is need for Health education & proper intervention programs.

A large number of women live half of their lives in a hormone deficient state without knowing how to alleviate this midlife phenomenon. The duties of health care professionals concerning menopause should be supportive, informative and interactive where women feel free to share their anxieties. Menopausal symptoms adversely affect quality of life. Hormone Replacement Therapy (HRT) is the most effective treatment. In spite of this, the risk-benefit ratio has always been debated. There is reduction in its use followed the report of Women's Health Initiative. Independent research shows that the continued negative attitude to the use of HRT for symptom relief is not justified. Treatment should be an individual decision of women after correct information and advice from healthcare professionals has been given.

RELATION OF THYROID CANCER AND ANTHROPOMETRIC FACTORS IN PAKISTAN

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Introduction and Objective: We have investigated the relationship of thyroid cancer (TC) and anthropometric factors in Pakistani population as this association remains to be controversial.

Methods: A total of 172 TC patients admitted at Aga Khan University Hospital (AKUH) from 2000–2014 were included. Patient's demographics, tumor subtypes, body weight, height, body mass index (BMI), body surface area (BSA) and body fat percentage (BF%) were calculated.

Results and Discussion: Out of 221 patients, 172 patients were included in this study. Mean age was 43 years \pm 15.40. Among all subtypes of TC, papillary remains the most common (72.1%), followed by medullary (10.5%), follicular (7.6%) and others (9.9%). Excess BMI $>$ 23 kg/m², BF%, BSA, weight and height were significantly associated with TC incidence in overall population (*p*-value $<$ 0.01*). In women, BF% was found to be in obese range (mean=35.07% \pm 7.6, *p*-value= $<$ 0.01*). In men, height $>$ 160cm and weight $>$ 60kg were positively associated with TC. No significant relation was seen between gender and BMI.

Conclusions: Excess BMI $>$ 23kg/m² and age $>$ 40years were positively associated with TC. In women, increased BF% and in men, height $>$ 160cm and weight $>$ 60kg had a significant relation with TC incidence.

EXPRESSION ALTERATIONS OF METABOLIC PROTEINS IN SCHIZOPHRENIC BRAIN REGIONS

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Introduction: Schizophrenia (SZ), a multifactorial disorder exhibits diverse neuropathological aberrations including defective neurotransmission system, neuroanatomical abnormalities and impaired synaptic connectivity. Along with these complications there is surfacing evidence that metabolic dysfunction and energy deficit may also contribute to its pathophysiology.

Methods: Present study entails, differential proteomic analysis of autopsied schizophrenic brain substantia nigra, cortex, and hippocampus (n=7) by using two dimensional electrophoresis (2DE) followed by Orbitrap mass spectrometry identification. Differential expression was validated by western blot, while In Silico analysis was applied for functional interactive role in metabolism.

Results: Proteome mapping of SZ patients revealed more profound alterations in SN protein expression than other brain regions compared to controls. SN exhibits differential expression of six proteins, enriched in several energy metabolic pathways including decreased expression of phosphoglyceratemutase 1, ATP synthase subunit d, mitochondrial and malate dehydrogenase cytoplasmic, while increased expression of glyceraldehyde-3-phosphate dehydrogenase, 4-trime-thylaminobutyraldehyde dehydrogenase, and alcohol dehydrogenase were found as compare to normal brain. Cortex and hippocampus also portray significant variations in expression patterns of five proteins across brain of schizophrenia patients.

Conclusions: Identification of differentially expressed metabolic proteins provides an insight for a better understanding of schizophrenia pathophysiology. Further characterization, will elucidate the functional mechanisms underlying defective

metabolic pathways, which will provide promise for future therapeutic developments in the treatment of schizophrenia.

ANALYSES OF PRE-MRNA SPLICING MACHINERY BY UV-INDUCED PROTEIN-RNA CROSS-LINKING AND MASS SPECTROMETRY

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Introduction and Objectives: The human genes that encode proteins undergo pre-mRNA splicing, an essential step of gene expression in which noncoding sequences are removed and coding sequences are ligated together. Unraveling splicing mechanism at the molecular level is not only important for understanding gene expression, but it is also of medical relevance, as aberrant pre-mRNA splicing is the basis of many human diseases or contributes to their severity. Although methods based on high-throughput sequencing have advanced our ability to identify the specific RNAs bound by a particular protein, there is a need for precise and systematic ways to identify RNA interaction sites on proteins.

Methods: We have developed an experimental workflow combining UV-induced cross-linking and high-resolution mass spectrometry for the identification of protein-RNA cross-linking sites. Purified complexes were UV irradiated at 254nm and proteins therein hydrolyzed with trypsin. Peptide-RNA cross-links were enriched by TiO₂ chromatography and analyzed by ESI-MS. Data analysis for identification of putative cross-links was performed by novel RNPxl pipeline.

Results: The workflow not only enables the systematic identification of cross-linked peptides but also amino-acid residues in RNA-interacting domains of proteins.

Conclusions: Successful identification of several cross-linking sites from human and yeast splicing complexes make it a promising technique to study any RNA-protein complex of interest.

CROSSTALK BETWEEN CYTOSKELETAL AND METABOLIC PROTEINS: A ROADMAP TO AD PATHOLOGY

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Introduction and Objectives: Cellular activities are based on complex networks of protein interactions. Identification and interaction analysis of associated protein partners is the basis of many biological studies, determining the key etiological factors. Alzheimer's disease, a pathologically complex and multifactorial disease is the most common cause of dementia. It is characterized by a progressive decline in memory and cognitive functions. Aberration of multiple pathways is observed in Alzheimer's disease including metabolic pathways which subsequently alter the allied cytoskeletal system. This study is designed to unveil the cytoskeleton and metabolic proteins interaction in AD and interpret their association.

Methods: Human autopsied brain tissue samples from normal age matched control (n=6) and AD patients (n=5) were procured. Two dimensional Blue Native/SDS PAGE combined with Orbitrap mass spectrometry analysis was accomplished to determine the interacting cytoskeleton and metabolic proteins. The interaction was further confirmed by co-immunoprecipitation.

Results: A total of thirteen protein complexes were obtained on BN-PAGE which resolved into many crucial proteins of different cellular networks. An important observation was a complex comprised of novel interacting partners including glyceraldehyde-3-PO4 dehydrogenase, actin cytoplasmic, microtubule associated

protein 1B and glial fibrillary acidic protein. Further the interaction between actin and GAPDH confirmed by co-immunoprecipitation has not been established before in AD.

Conclusion: In accordance with the previous findings of GAPDH and actin differential expression and post translational modification, our results suggest interesting implications of these two proteins in AD.

DETECTION OF HIGH RISK HUMAN PAPILLOMA VIRUS (HPV) GENOTYPES 16/18 IN ORAL LESIONS OF TOBACCO CHEWERS IN PAKISTAN

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Introduction and Objective: The Human Papilloma Virus (HPV) has evolved as a new culprit of malignant and premalignant oral lesions. The objective of this study was to find out the frequency of HPV and its high risk genotypes in different lesions of oral cavity of tobacco chewers.

Methods: From 492 subjects (421 males and 71 females), 20 ml of oral rinse sample was collected after obtaining an informed consent. Normal subjects with no chewing habits (250) including 135 males and 115 females were also taken from same setting. Gentle brushings over the lesions with the help of dental floss brush and stored at 4°C until DNA extraction. DNA was extracted and PCR was performed using HPV consensus primers Gp5+/Gp6+ and HPV 16, 18 specific primers for genotyping. Categorical data was calculated as frequencies and percentages.

Results: Oral pre-malignant lesions were present in 421 (86%) males and 71 (14%) females having leukoplakia (173, 35%), erythroplakia (60, 12%), submucous fibrosis (192, 39%) and L/E (67, 14%). Total number of HPV positives were 128 (26%), having HPV 16 (13%) and HPV 18 (11%) whereas, 76% had other genotypes. Among submucous fibrosis 82 (46%) were HPV positive. Out of total 128 HPV patients 92% were males and 8% were females. All controls were found to be HPV negative.

Conclusion: Frequency of HPV was found high (26%) in oral lesions with HPV16/18 as 13% and 11% respectively. The patients with submucous fibrosis are at greater risk of having HPV. Other HPV genotypes causing premalignant lesions require further investigation.

DIAGNOSTIC, PROGNOSTIC AND PREDICTIVE VALUE OF MICRORNA-21 IN BREAST CANCER PATIENTS, THEIR DAUGHTERS AND HEALTHY INDIVIDUALS

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Introduction: MicroRNA-21 (miR-21) located on 17q23.1 has shown high expression in breast cancer. This gene has anti-apoptotic ability and causes tumor cell growth. It is also normally involved in functions such as signal transduction pathways affecting normal cell growth and differentiation.

Objective: To determine the presence of miR-21 in the serum levels of breast cancer patients, their daughters and healthy individuals.

Methods: A total of 132 subjects were recruited: 50 breast cancer (cases), 50 age-matched healthy individuals (control A) and 32 daughters of index cases (control B). The sampling technique used for cases was random sampling. Controls were selected by purposive method. Serum tests were run on qRT PCR and threshold cycle was determined and fold change calculated.

Results: Fisher exact test was used when analyzing between cancer patients and healthy patients or with their daughters. Normality of continuous variables was assessed by Shapiro Wilk's test. While comparing the same between two groups, student t-test and Mann-Whitney test was used. P-value ≤ 0.05 was considered significant. MiR-21 was significantly higher in cases as

compared to control A and B (p-value=0.001). However control B showed significant gene expression as compared to control A (p-value=0.001). The $2^{-\Delta\Delta Ct}$ formula was applied to compare the fold change between the groups. The cases were also divided into ER, PR and HER2 positive and negative cases. It was observed that triple negative cases showed a greater expression of gene as compared to other groups (p-value=0.001).

Conclusion: High expression of miR-21 in breast cancer patients suffering from stage III invasive ductal carcinoma had been calculated as compared to its age matched healthy subjects. This result can mark miR-21 as a potentially strong diagnostic and prognostic biomarker of breast cancer. MiR-21 profile was also studied in daughters of the index cases. Their expression was also shown to be significantly higher as compared to the healthy individuals but lesser than the full blown disease of breast cancer. This result strengthens the concept of inheritability of this disease and this gene can also be labeled as a predictive biomarker. When Ct-values and fold change calculations were compared between ER, PR and HER2, higher expression was seen in negative tumor tissues which are very resistant tumors and respond poorly to chemotherapy.

SERUM METABOLOMIC PROFILES FOR BREAST CANCER DIAGNOSIS, GRADING AND STAGING BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Introduction and Objectives: Detection of metabolic signature for breast cancer (BC) has the potential to improve patient prognosis. This study identified potentially significant metabolites differentiating between breast cancer patients and healthy controls to help in diagnosis, grading, staging and determination of neoadjuvant status.

Methods: Serum was collected from 152 pre-operative breast cancer (BC) patients and 155 healthy controls in this case-control study. Gas chromatography-mass spectrometry (GC-MS) was used to obtain metabolic profiles followed by chemometric analysis.

Results: Chemometric analysis showed the identification of significantly differentiated metabolites including 7 for diagnosis, 19 for grading, 23 for staging, 15 for stage III subcategories and 10 for neoadjuvant status (p value < 0.05). Partial Least Square Discriminant Analysis model revealed a distinct separation between healthy controls and BC patients with a sensitivity of 80% and specificity of 100% on external validation. Models for grading, staging and neoadjuvant status were built with Decision Tree Algorithm with predictive accuracy of 71.5%, 71.3% and 79.8% respectively.

Conclusion: Pathway analysis revealed increased glycolysis, lipogenesis, and production of volatile organic metabolites indicating the metabolic alterations in breast cancer.

GROWTH FACTORS MEDIATED PRECONDITIONING OF MESENCHYMAL STEM CELLS: STRATEGY FOR ENHANCING RENAL TISSUE REGENERATION

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Introduction and Objectives: Acute kidney injury (AKI) is a complex disorder manifested by rapid loss of renal function. AKI has strong influence on expression of renal specific genes which may play central roles in key cellular pathways and targeting these pathways may turn out to be effective therapeutic option. Transplantation of mesenchymal stem cells (MSCs) has been shown to improve kidney function following injury. However, poor survival and grafting of stem cells to the site of injury has restricted their therapeutic efficacy. Preconditioning of stem cells can be a potential strategy; chemokines and growth factors can improve their potential in terms of homing and differentiation. Objectives of the present study were to examine the changes in the expression profile of genes associated with renal injury and to explore the novel strategy of preconditioning of MSCs for injured kidney cells.

Methods: Rat AKI model was established with gentamicin and renal specific genes were analyzed through RT-PCR. For preconditioning strategy we used IL-7 gene to transfect MSCs. MSCs were co-cultured with cisplatin-treated injured Mardin-Darby bovine kidney (MDBK) cells and their in vitro fusion potential was analyzed.

Results: Several genetic changes occur in AKI that may contribute in long term renal injury consequences and targeting these pathways may appear to be effective therapeutic options. Flow-cytometry of preconditioned MSCs and injured MDBK cells revealed significant ($P \leq 0.001$) cell fusion compared to that of the normal MSCs. In addition, we also observed improved migration ability of preconditioned MSCs in the in vitro wound healing assay.

Conclusions: For efficient stem cell therapy, it would be valuable to determine the contribution of various cellular factors in the fusion process so that a rationale for the use of such preconditioned cells can be determined.

AMINO ACID RACEMIZATION IN HUMAN DENTINE AS AN INDICATOR OF CHRONOLOGICAL AGE – A STUDY IN KARACHI, PAKISTAN

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Introduction and Objectives: Amino acid racemisation is a reliable method to estimate age in developed countries. This study was designed to determine the coefficient of amino acid racemisation (AAR) with reference to age in our population. The objectives are to determine the coefficient of aspartic acid racemisation (AAR) with reference to age by HPLC, to measure the mean error in calculating age using this method; and to identify any differences from earlier observation and discuss the reproducibility in our setting.

Methods: Hundred (100) teeth were obtained from subjects between the age of 11 and 70 years who had come for routine extraction procedure or for orthodontic procedures. Incisors, canines, premolars and, first and second molars were included. Carious tooth and third molar were excluded. Samples were stored, dried, de-mineralized, hydrolyzed, and derivatised. High-Pressure Liquid Chromatography (HPLC) was performed to quantify the L- and D-forms of aspartic acid in dentin. Correlation and regression was then tabulated based on the quantification, and was compared with data from other studies.

Results: It was observed that there is very strong positive correlation (0.818) observed between actual age and co-efficient of racemization (statistically significant at 5%), which indicates that as age increase co-efficient of racemization increases. Based on the analysis of 85 samples to estimate age least Square method was used to derived the regression line i.e. Age = $-4.391 + 347.396$ (co-efficient of Racemization). Co-efficient of determination was found to be 0.74, which show that regression equation for estimating age was 74% correct. The computed regression line was tested by using coefficient of racemisation for 15 samples, we observed that the

mean of actual age (38.44 ± 13.22) was not significantly different from the mean of calibrated age (37.52 ± 12.36) which is estimated by regression equation (P -value = 0.222). The mean error (difference between actual and calibrated age) is found to be 0.911 ± 7.41 .

Conclusions: The correlation coefficient was strongly positive. The results strongly suggests that aspartic acid racemisation of human dentine is a precise method for estimation of chronological age in living and dead. The methodology should be standardized to make the results more accurate and prevent fallacies. However, it remains an expensive procedure, considering that the laboratory equipment is not available in most cities of Pakistan.

CORRELATING VITAMIN D DEFICIENCY WITH VOLUMETRIC BREAST DENSITY VIA A FULLY AUTOMATED SOFT WARE VOLPARA™ IN THE REPRODUCTIVE AGE GROUP

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Objective: To see the correlation of volumetric breast density and Vitamin D deficiency in the reproductive age group.

Methods: We enrolled 300 patients from the general surgery outpatient department, their blood samples were collected and vitamin D level was determined via chemiluminescence Method. Full field digital mammography was done and the DICOM images were tagged and processed via fully automated software Volpara™ by using the formula " $\text{Volumetric breast Density} = 100 \times \text{Volume of fibroglandular tissue (cm}^3\text{)} / \text{Volume of breast tissue (cm}^3\text{)}$ ".

Results: There is enough evidence now that Vitamin D deficiency is inversely related to mammographic density and MD itself is a strong predictor of breast cancer risk. Measurement of volumetric breast density objectively via a fully automated software "Volpara" and using the Raw Processed version of the FFDM in this study was attempted for the first time in Pakistan and correlating with the vitamin D deficiency and also BI-RADS (Breast-Imaging reporting and Data system) which is a common method used in clinical practice worldwide. The total number of patients had only two women with normal vitamin D level, the deficient group was evaluated. In the group of women with low Vitamin D (Vitamin D < 50), 76% had elevated VBD and 24% had normal VBD. The test for proportions compares whether these two groups are significantly different in size or not.

Conclusions: The null hypothesis assumes that vitamin D deficient women have the same number of people in tolerable and excess groups. Because the p-value is small, the null hypothesis is rejected and we conclude that the number of women with elevated breast density is not the same as the number of women with normal breast density, it is higher.

NATURAL COMPOUNDS SUPPRESS OXIDATIVE STRESS AND INFLAMMATORY RESPONSE IN ACTIVATED MOUSE MACROPHAGES

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Introduction: Inflammation is quite complex, initiated by several factors includes, molecules ranges from bacteria to chemical causing cellular trauma. Tissue injury induced by this trauma results in the inflammatory mediators release by macrophages, including reactive oxygen species (ROS) like superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), nitric oxide and increased expression of pro-inflammatory cytokines, NF kappa B, and inducible Nitric Oxide Synthase. A current study has led to the isolation of two new Acylphloroglucinols derivatives along with four known compounds, Myricetin, Icosonic acid, G3-factor and

myrtocommulone E from *Myrthus*.

Methods: In this study, we used Lipopolysaccharide induced macrophages to study immune mediators release in culture supernatant. We also sought to further delineate the underlying mechanisms elicited by oxidative stress and inflammation and suppressed by compounds.

Results: The results show that new Acylphloroglucinols derivatives and Myricetin significantly suppressed the oxidative stress and anti-inflammatory efficacy in suppressing, total reactive species (ROS), superoxide, nitric oxide, pro-inflammatory inducible nitric oxide synthase (iNOS), proliferation of T-cells and nuclear factor-kappa B (NF- κ B) nuclear translocation. All the compounds were also non toxic to other cell systems.

Conclusion: Based on these results, we conclude that natural product may contribute in anti-inflammatory drug discovery.

IMIPENEM RESISTANT PSEUDOMONAS AERUGINOSA: THE FALL OF THE FINAL QUARTERBACK

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Introduction and Objectives: To isolate, determine the frequency, and study the demographic trends of MBL positive *Pseudomonas aeruginosa* from imipenem resistant isolates collected from clinical samples in a tertiary care hospital of Pakistan.

Methods: In this cross sectional study a total of 230 strains of *Pseudomonas* were isolated from various clinical specimens on the basis of culture and biochemical tests. Imipenem resistant isolates were selected by Kirby Bauer Diffusion technique, followed by screening for MBL production by Imipenem EDTA Combined Disk Test. Demographic details of each patient were recorded on a separate questionnaire. Chi-Square goodness-of-fit test was computed to review the isolation of MBL positive isolates (P-value \leq 0.05) in different specimen.

Results: Out of 230 strains of *P. aeruginosa* 49.5% were imipenem resistant; MBL production was confirmed in 64.9% of the resistant isolates. Resistance to polymyxin B (12.5%) was notable. Majority of the MBL positive strains were isolated from patients aged between 20–39 years (45.9%) and the predominant source was pus (43.24%) which was found to be statistically significant (P-value=0.04). Outpatient departments (24.3%) and burn unit (21.6%) were the major places for resistant isolates.

Conclusions: MBL production is one of the major causes of IRPA. Increasing resistance to polymyxin B is grave. Due to acquisition of MBL strains, MDR *P. aeruginosa* has become endemic in tertiary setups.

ANTIBIOTIC RESISTANCE IN NEONATAL SEPSIS – STUDY ON LOW BIRTH CASES FROM KARACHI-PAKISTAN

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Introduction and Objectives: Premature neonates are often immunocompromised and readily susceptible to infectious diseases. They are deficient in antibodies whereas neonatal neutrophils are immature and depleted (2 fold in circulatory pool). Neonatal anatomic barriers are susceptible and hence infection control poses significant challenge. The objective of this study is to assess the common pathogens isolated in neonatal sepsis and specify the antibiotic resistance pattern.

Methods: The study was conducted in the neonatal ICU of two public sector tertiary care hospitals inclusive of 234 cases. Premature neonates with clinically confirmed bacterial infection were included comprising of 48.6% cases of Early onset neonatal sepsis (EONS) with obstetrical complication and fulminant, multisystem

disease. Intrapertum fever was recorded in 87% cases of EONS among which, 22% culture positive neonates were initially asymptomatic. Apnea, fever and metabolic acidosis were the more prevalent symptoms. CBC, C-reactive protein, Glucose and Blood culture test were recorded.

Results: The most common pathogen was Group B streptococci (24%), *Klebsiella pneumonia* (21%) and *E.coli* (15%). Fetal hypoxia was confirmed in 34% cases. Ampicillin and Gentamycin was given empirically in 87% cases. Modified Kirby Baur disc diffusion technique was used to determine antibiotic resistance. *Klebsiella pneumonia* was greatly resistant against cephalosporins (average of 54%) and co-trimoxazole (87.9%) whereas comparatively susceptible to quinolones (ciprofloxacin). Resistance of amikacin was elucidated in 87% cases.

Conclusion: In lieu with preventive approaches to avoid neonatal sepsis, careful treatment choice of antibiotic is required to deplete chances of therapeutic failure.

WITHANIA COAGULANS: A POTENTIAL CHOLESTEROL LOWERING PLANT

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Introduction and Objective: *Withania coagulans* has many medicinal properties. Currently its cholesterol lowering effect was investigated in terms of HMG-CoA reductase inhibiting activity.

Methods: The study was designed to investigate in-vivo effect of methanolic fruits extract (MFET) of *W. coagulans* in hyperlipidemic rabbits. On the completion of trial, total cholesterol (TC) and HMG-CoA/mevalonate ratio were estimated. Additionally, in-silico study was performed to find out the inhibitory effect of 21 chemical compounds present in fruits of same plant on HMG-CoA reductase (HMGR). Molecular docking calculations were carried out by using Molegro Virtual Docker (MVD) software.

Result: All doses of MFET were significantly (p < 0.05) decreased TC and improved HMG-CoA/mevalonate ratio indicating HMGR inhibition. Interestingly, 5 compounds were displayed the highest MolDock score including Ergosta-5, 25-diene-3 β , 24 α -diol, Withacoagulin, Withanolide D, Coagulin D and Withaferin. Of which, excluding Withanolide D rest of the 4 compounds interacted with catalytic residues present in the active site of HMGR by forming hydrogen bonds and inhibited the activity of same enzyme.

Conclusions: The present in-vivo and in-silico studies proved that compounds present in fruits of *W. coagulans* are predicted as potent inhibitors of HMGR that could be used for the development of drug for hypercholesterolemia.

CARDIOPROTECTIVE EFFECT OF MODIFIED BONE MARROW MESENCHYMAL STEM CELLS AFTER TRANSPLANTATION IN RAT MODEL OF MYOCARDIAL INFARCTION: AN APPLIED APPROACH TOWARDS CELLULAR THERAPY

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Introduction and Objectives: Myocardial infarction is increasing cause of death worldwide. In South Asia, cardiac diseases are increasing at a rate greater than in other part of the world. Regenerative medicine involves the repair or regeneration of an organ, tissue or cells in order to restore an impaired function of the tissue. The cellular therapy offers a promising therapeutic strategy for the treatment of different diseases including myocardial infarction. Mesenchymal stem cells (MSCs) are having immense importance in the emerging field of regenerative medicine and have tremendous capability for cellular therapy and tissue engineering. MSCs can be converted into cardiomyocytes by treating

them with DNA demethylating agents. Our aim is to investigate the cardioprotective effect of pretreated MSCs when transplanted in infarcted myocardium of rat.

Methods: In the present study, rat mesenchymal stem cells were treated with DNA-demethylating agents. The optimized concentrations of these compounds were added separately into the culture medium and the treated cells were analyzed for the expression of cardiac specific genes by RT-PCR and cardiac specific proteins by immunocyto-chemistry and flow cytometry. The treated MSCs were then transplanted to the rat model of myocardial infarction and cardiac functional studies were performed by echocardiography.

Results: We have found expressions of cardiac specific proteins and genes in the modified mesenchymal cells both at genetic and protein levels. Transplantation of modified MSCs into infarcted myocardium of rat improved the cardiac left ventricular function possibly by enhancing the rate of bone marrow stem cell differentiation into mature cardiomyocytes in the infarcted heart.

Conclusions: We therefore, conclude that pre-treating autologous mesenchymal stem cells before transplantation may increase the likelihood of successful regeneration of damaged myocardium. The study put forth another valuable aspect that would serve as a tool for modified cellular therapy.

ANTIPLATELET AGGREGATING AND MEMBRANE STABILIZING PROPERTIES OF ARECA NUT

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Introduction and Objectives: Platelet aggregation inhibitors are one of the key preventive and therapeutic agents for cardiovascular diseases. Areca nut is used in folk medicine for treatment of various ailments. Present study was conducted to investigate the antiplatelet aggregating and membrane stabilizing activities of areca nut extract and its fractions using platelet aggregation and membrane stabilization assays.

Methods: To determine the effect of areca extract and its fractions on platelet aggregation, blood was taken from healthy human volunteers and platelet aggregation was monitored. Membrane stabilizing properties were assessed using human red blood cells.

Results: Our results showed that areca extract was effective against arachidonic acid induced platelet aggregation in dose dependent manner leading to complete inhibition at 10 mg/ml dose. Data obtained from the present study showed that the areca extract (EC₅₀= 91 µg/ml) and its aqueous fraction (EC₅₀= 55 µg/ml) possess significant membrane stabilizing activity in a dose dependent manner (IC₅₀= 100 µg/ml).

Conclusions: These findings suggest that areca nut extract, ethylacetate fraction and aqueous fraction possess significant antiplatelet aggregating and membrane stabilizing properties, and aqueous fraction was most effective which may be of clinical value. However, further studies are required to identify its active compound(s) via bioassay guided-fractionation method.

SMALL MOLECULAR ACTIVATORS OF PROTEASOME-RELATED HSLV PEPTIDASE

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Introduction and Objectives: The HslVU or prokaryotic mini-proteasome is two component system based on HslV peptidase and HslU ATPase. Like proteasome it is involved in intracellular proteins degradation. Due to its occurrence in pathogenic microbes and absence in human beings, it is considered as a potential antimicrobial drug target. The functional HslVU complex forms

when HslV dodecamer is flanked at both sides by HslU hexamers and the carboxy termini of HslU subunits intercalate into a clefts between two adjacent HslV subunits. This intercalation is followed by conformational change in HslV active site resulting in its allosteric activation. We for the first time report certain small molecules which are capable of activating HslV peptidase in the absence of its natural activator, HslU.

The benzimidazole, quinazoline and chromone derivatives were suggested by ligand docking studies to bind at the carboxy terminus binding pocket of HslV in a manner similar to HslU carboxy terminus. This was confirmed by HslV activation assays with these compounds that gave ED₅₀ in micromolar range (0.6–1.5 µM).

Results: The results showed that small, extracellular non-peptidic molecules can allosterically activate the peptide hydrolytic activity of HslV, which in turn would initiate intracellular proteolysis.

SENSITIVITY PATTERN OF MORAXELLA CATARRHALIS ISOLATED FROM HOSPITALIZED PATIENTS

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Introduction and Objectives: *Moraxella catarrhalis* (formerly known as *Branhamella catarrhalis*) has emerged as a significant bacterial pathogen of humans over the past two decades. Over the same period, studies have revealed its involvement in respiratory (e.g., sinusitis, otitis media, bronchitis, and pneumonia) and ocular infections in children and in laryngitis, bronchitis, and pneumonia in adults. It has discovered an increasing number of B-lactamase-positive strains. We aim to find out the sensitivity pattern of *Moraxella catarrhalis* isolated from sputum.

Methods: Total 124 sputum samples were analyzed. Confirmation done by using chocolate agar a typical golden yellow which can be distinguished by hockey puck like colony. Further identifications were done by gram staining, oxidase test and biochemical test. Antibiogram was done by CLSI method. Statistical analysis was also performed using SPSS version 17.

Results: Sensitivity pattern showed AMC 99%, TE 49%, CRO 01%, E 30%, SXT 04%, CIP 50%, CEF 84%.

Conclusions: To control the resistivity of these organisms some preventive measurements like proper identification and selective medication should be taken.

ASSESSMENT OF HCV GENOTYPES GENE FLOW HYBRIDIZATION AND ITS COMPARISON WITH CONVENTIONAL PCR

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Background: Nucleotide sequence analysis of hepatitis C virus (HCV) strains showed substantial variability leading to a classification into several genotypes and subtypes.

Objective: The objectives of the present study were the knowledge of the HCV genotypes in Pakistan, and determining their prevalence.

Methods: During the course of this study, HCV-RNA positive sera samples from 200 chronically infected patients were characterized by genotyping assay and Automated Sequence Analysis. Genotyping assay utilized type-specific primers for amplification of the core region, where as sequencing was done for 5'Noncoding region of HCV using ABI 3100 Sequencing Analyzer.

Results: During our study we came across that genotype 3a was the most prevalent (36%) followed with 3b (26%), 3c (3.5%), 3d (4%), 1a (2.5%), 1b (1%), 1c (2.5%), 1d (0.5%), 2a (12%), 2b (0.5%), 2c (0.5%), 4 (0.5%) and 4a (1.5%). Genotypes 3d, 1c, 1d and 2c were reported for the first time from Pakistan

Conclusion: Advances in the field of molecular biology have provided rapid diagnostic tools that have reduced the turnaround

times for detecting HCV genotype by using “Flow-through” hybridization in Pakistan.

GALECTIN-3 AND WNT SIGNALLING IN MYOCARDIAL INFARCT HEALING: IS THERE A CONNECTION?

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Introduction and Objectives: Myocardial infarction (MI) leads to complex repair processes including scarring and fibrosis. Galectin-3 (Gal-3) is a beta-galactoside binding lectin responsible for cardiac fibrogenesis and adverse left ventricular (LV) remodeling. Canonical Wnt signaling in myofibroblasts following MI also modulate wound healing and fibrosis. As Gal-3 and Wnt 3a play a significant role in the post MI sequel we aim to measure their concentration in murine heart at 7 week post Ischemia/reperfusion injury and to determine their correlation.

Methods: Male C57B6/J mice were used for myocardial IR injury by temporarily ligating the left anterior descending artery of the heart for 30 minutes to create ischemia/infarction followed by restoration of blood flow. Samples collected 1 week post IR injury point was processed for ELISA to detect Gal-3 and Wnt-3a levels in the heart.

Results: Gal-3 and Wnt 3a levels were significantly increased in the LV heart tissue at 1 week post IR compared to sham operated mice. There was a strong and significant positive correlation observed between Gal-3 and Wnt-3a at 1week post IR injury.

Conclusions: Gal-3 and Wnt-3a levels were increased in 1 week post myocardial IR injury in conjunction with intrinsic cardiac repair. Understanding the mechanisms of this repair process could lead to optimal management and prevention or delay in the onset of heart failure.

STD PATHOGENS DETERMINED IN SEMEN USING PCR AND “FLOW-THROUGH” HYBRIDIZATION TECHNOLOGY

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Background: The prevalence of sexually transmitted Disease (STDs) in health care facilities for diagnosis of common pathogens, those causing infertility like Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma hominis. Genital wart is a highly contagious sexually transmitted disease caused by some sub-types of human papillomavirus (HPV).

Methods: Semen samples were obtained by masturbation into sterile containers after sexual abstinence of 48 to 72 hours. Samples were subjected to semen analysis within one hour of collection. The concentrations of sperm as well as sperm motility were also determined. DNA extraction was extracted of all the samples and the PCR assay was performed. The amplicons are subsequently hybridized to pathogen-specific capturing probes via “Flow-through” hybridization.

Result: During our study we came across with the STI pathogens present in our population and the reason for infertility was the main cause. When Chlamydia trachomatis and Neisseria gonorrhoeae were detected in their wife’s were also screened and these STI pathogens were identified.

Conclusions: The main route for the transfer of STI pathogens were the men special those who visited commercial sex workers as they were working in other cities and the complained for infertility. Screening for bacterial STI pathogens, Mycoplasma hominis, Chlamydia trachomatis and Neisseria gonorrhoeae are strongly recommended because these pathogens can cause serious reproductive complications.

EFFECT OF CITRUS PARADISI ON COAGULATION MECHANISM

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Introduction and Objectives: Over the past few years, herbal remedies and medicinal plant entities are of great interest in various region of the world for their therapeutic and preventive effects in various cardiovascular ailments like atherosclerosis. Numerous animal models were studied extensively for elucidation of these plant component effects on different organs. Citrus Paradisi (*C. paradisi*) due to its antioxidant and anti-inflammatory potential has been selected in this study to evaluate the effect of this compound on coagulation and anticoagulation factors. *C. paradisi* contains high concentrations of hesperidin, naringin, limonene and related flavonoids.

Methods: Doses were selected in the range of 0.1-0.5 ml/kg. Aspirin and warfarin 150mg/kg and 5mg/kg-10mg/kg respectively were used as standard anticoagulants. Animals were divided into six groups with ten rabbits in each group.

Results: At median dose level, significant enhancement in thrombin, pro-thrombin time was observed in comparison to control, while fibrinogen levels were significantly reduced with warfarin. Furthermore, substantial inhibition of platelet aggregation by adenosine phosphate, collagen, epinephrine and arachidonic acid was observed at median *C. paradise* dose (MCPD).

Conclusions: Results of this study have shown an effective anticoagulant activity. Thus the patients, who are at risk of cardiovascular event development may additionally be advised to consume grape fruit juices in their diet to reduce the chances of mortality and associated morbidities.

CONVENTIONAL CLINICAL AND PROGNOSTIC VARIABLES IN 150 ORAL SQUAMOUS CELL CARCINOMA CASES FROM THE INDIGENOUS POPULATION OF KARACHI

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Objectives: To evaluate conventional clinical and prognostic variables in Oral Squamous Cell Carcinoma (OSCC) cases from the indigenous population of Karachi.

Methods: Common clinical and pathological parameters related to poor prognosis of 150 OSCC cases presented at two major treatment facilities of Karachi were evaluated. The reporting included demographic details and variables like intra-oral subsites, clinical stage and histological grades. Recurrence of tumor after initial resection was also documented.

Results: The patient’s population comprised of 98 males and 52 females. The mean age was 47.1 + 12.22 (range: 20-78 years). Maximum numbers were seen in the 41–50 years age group. Urdu-speaking community was the most affected ethnic group (n=75). Histopathological analysis revealed that majority of cases was moderately differentiated tumors (59%) with clinical stage II (35%) or IV (29%). The most common intra-oral subsite came out to be buccal mucosa of cheeks (56%) followed by lateral borders of tongue (21%), lips (13%), alveolar (6%), palate (2.6%) floor of mouth (1.3%), etc. Recurrence was observed in 8 out of 150 cases. All patients underwent primary resection ± neck dissection and reconstruction where possible.

Conclusions: Overall experience with oral squamous cell carcinoma shows that it has a high tendency for local invasion as well as dissemination to regional lymph nodes, i.e. cervical lymph nodes, both are associated with a poor prognosis. Preventable risk factor of tobacco chewing has been observed in majority of these cases.

Poster Presentations

ROLE OF MUSCARINIC G-PROTEIN COUPLED RECEPTOR (GPCR) IN PROSTATE CANCER GROWTH AND SURVIVAL

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Introduction: Despite advances in early detection and treatment, prostate cancer remains one of the most frequently diagnosed cancers and a leading cause of cancer death in males worldwide. Much work has been done on androgen receptors in relation to proliferation and survival, and it has been shown that cancers of this organ usually turn androgen insensitive and resistant to therapy. There has been very little, if any, work done on the precise contribution of cell surface muscarinic GPCRs in prostate cancer growth.

Methods: Here we determined the role of the M1 receptor in prostate cancer cell proliferation. The treatment of PC-3 cells with selective M1 receptor antagonists, Dicyclomine exhibited anti-proliferative effects using growth assays, while M1 receptor agonist, Pilocarpine exhibited increased proliferation compared with the untreated cells.

Results and Conclusions: Further studies will dissect molecular pathways to determine the role of the M1 receptor in androgen sensitive and androgen insensitive prostate cancer cell growth and proliferation. The results of this work may help inform clinical and public health interventions for prostate cancer by providing insights into the biology of prostate cancer progression.

CO AND CONTRA-REGULATION OF GLYCOSYLATED PROTEINS IN THREE DISTINCT REGIONS OF SCHIZOPHRENIC BRAIN

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Introduction and Objectives: Schizophrenia, a multifactorial disorder exhibits diverse neuropathological aberrations with altered protein expression and post translational modifications as a surfacing evidence that may also contribute to its pathophysiology. To investigate the integrated picture of molecular changes and potential alterations in expression of specific glycosylated proteins in different brain regions compared to physiologically normal brains. We aimed to provide more holistic view of three brain regions; and their dynamic cross-talk providing insights into the underlying molecular mechanisms associated with schizophrenia.

Methods: Present study entails, differential proteomic analysis of autopsied brain regions of schizophrenic; substantia nigra, cortex, and hippocampus (n=7 each), by using sodium dodecyl sulphate polyacrylamide gel electrophoresis coupled with immunoblot and DIG (digoxigenin) labelling followed by ESI-QTOF MS analysis for validation.

Results: We have identified fourteen glycosylated protein components with altered expression among the brain regions. The 50KDa (Glial fibrillary acidic protein, GFAP) and 84KDa (mitochondrial inner membrane protein) are contra-regulated between substantia nigra and cortex. While T-complex protein 1 subunit zeta of 58KDa is co-regulated between substantia nigra and hippocampus. The remaining five proteins identified with significantly altered glycosylation intensity in the specific brain regions give evidence of their explicit regional function. Additionally, nine proteins commonly appeared non-glycosylated in the three studied brain regions.

EXPRESSION AND ASSOCIATION OF CDK10 WITH ETS2 DURING HUMAN CORNEAL WOUND HEALING

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Introduction and Objectives: Corneal related complications are major health concerns worldwide because its progression is associated with significant impaired vision. Therefore, there is an urgent need to develop reliable understanding of the underlying mechanism of corneal epithelial wound healing to apply

therapeutic options. We aimed to investigate the alterations in protein expression and association of CDK10 and ETS2 during corneal epithelial migration and to demonstrate the networks of the total identified proteins with potential dual functions.

Methods: In this study, human corneal epithelial cells lines (HCEC) have been used for wound healing model. Mechanical wound was made in HCEC lines and healing was monitored at 24, 48 and 72 hours of post wounding. Epithelium was scrapped at 24, 48 and 72 hours, followed by protein quantification using BCA kit. The wounded and unwounded cells were subjected to SDS-PAGE and two-dimensional electrophoresis (2DE). Mass Spectrometry (MALDI TOF) was done to identify the proteins through protein database searches. The identified protein were further analyzed and validated by western blot analysis. A further insight into the links among the identified proteins and their functional roles were analyzed by STRING 8.3 software.

Results: A significant finding of the present study is the identification of Cdk10, EFN3, RAB 34, RRAS, HSP2 and HSP90 in healing corneal epithelium at active phase of migration. Expression of CDK10 and ETS2 were validated using antibody by western blot. Interaction association network analysis further confirms the close interacting relationship between CDK10 and ETS2 proteins.

Conclusions: These findings are one step forward in identifying the mechanism of wound repair or re-epithelialization. Association of CDK10 and ETS2 in migrating samples suggested that they have positively regulated the cell cycle and cell proliferation and hence mediate the wound healing. This study may also increase the understanding of normal and abnormal corneal function with likely relevance to corneal disease and transplants.

FUNCTIONAL ASSOCIATION OF BAD AND 14-3-3 PROTEINS IN SCHIZOPHRENIC PATIENTS

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Introduction and Objectives: Schizophrenia is a multifactorial disease which involves the exogenous and endogenous interaction and is characterized by neuronal reduction, dendrite deficits, decreased synaptic markers and alteration in synaptic circuitry. Schizophrenia is a neurodevelopmental disorder but apoptosis may contribute to the pathophysiology of schizophrenia due to progressive clinical deterioration and subtle neurostructural changes following the onset of psychosis. Apoptosis is a mechanism of cell death, is regulated by a complex cascade of pro and anti-apoptotic proteins. Dysregulation of apoptosis in several cortical regions of schizophrenia indicates that apoptotic vulnerability is increased. Pro-apoptotic protein BAD (Bcl-2 associated death promoter) interacts with anti-apoptotic BCL-2 and inhibit its activity while 14-3-3s binds with BAD and inhibit apoptosis that it act as an ant apoptotic protein. Various proteomic based studies shown reduction of 14-3-3 proteins in schizophrenic brains. In this study we aimed to identify and validate the differential expression of BAD and 14-3-3s by western blotting and their association network with other proteins by String 10, Qiagen pathway data bases.

Methods: Expression of BAD and 14-3-3 proteins from autopsied schizophrenic brain cortex were validated by western blotting and In Silico analysis was done to see the association with other proteins by using String database, Qiagen pathway.

Results: We have identified differential expression of BAD and 14-3-3 proteins in cortex region of schizophrenic human brain by western blotting. Interaction association network between BAD and 14-3-3 (YWHAQ, YWHAH, YWHAE, YWHAB, YWHAZ, Y-WHAG) was analyzed by string software. Qiagen pathway further confirms the close interacting relationship between BAD and 14-3-3

proteins in apoptotic pathway related to schizophrenic patients.

Conclusions: Apoptotic proteins BAD and 14-3-3s association in Schizophrenic brain cortex region provide insight into the apoptotic pathways which play role in the pathophysiology of schizophrenia which could lead to development of therapeutic drugs to target apoptotic proteins and their interacting partners.

KISSPEPTIN AND UNEXPLAINED INFERTILITY

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Introduction and Objectives: To compare Kisspeptin (KP) levels in females with infertility to male, female and unexplained causes and associate it with success of treatment after ICSI.

Methods: Cross sectional study carried from August 2014 till May 2015 included all categories of infertility with duration > two years, female age between 20 to 50 years and body mass index > 18 < 35 kg/m². Down-regulation of ovaries was followed by calculated stimulation, ovulation induction, oocytes retrieval, ICSI, in vitro maturation of embryos and finally blastocysts transfer. KP levels were measured by enzyme linked immuno sorbent assay on day of ovulation induction. Failure of procedure was detected by beta human chorionic gonadotropin < 5–25mIU/ml (non-pregnant) whereas females with levels > 25mIU/ml and no cardiac activity had preclinical abortions. Clinical pregnancy group had confirmed cardiac activity on trans vaginal scan. Data was analysed using SPSS 15.

Results: Females who completed the procedure (168) were stratified; 40 (24%), 66 (39%) and 62 (37%) with male, female and unexplained infertility. The KP levels were 285.38 ± 23.96 ng/L (mean ±SD). The females with unexplained infertility had lowest KP (101.4 ± 11.96) which was statistically significant (p < 0.05). The results declared 66 (39%) females as non-pregnant, 30 (18%) with preclinical abortions and 72 (43%) with clinical pregnancies. Out of non-pregnant females 42 (64%) had unexplained cause of infertility.

Conclusions: Low KP levels in unexplained females explains the peripheral role of the neuropeptide, deficiency of e—which leads to failure of implantation after ICSI.

RELIABILITY OF NEPHRIN AS AN EARLY DIAGNOSTIC BIOMARKER FOR SCREENING DIABETIC NEPHROPATHY

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Introduction and Objectives: Diabetic nephropathy occurs as a result of proximal tubule dysfunction with podocyte damage due to increased advanced glycation end-products insults in diabetes. Nephritin, one of the three proteins that make up the podocyte architecture, is excreted foremost following renal damage. The aim of this study was to evaluate the efficiency and reliability of nephritin for an early biomarker of kidney damage in diabetic patients.

Methods: Urine samples (78) were collected from diabetic center. Protein and glucose were determined by Dipstick. The patients were grouped on the basis of Albumin/creatinine ratio (UACR) as normoalbuminuric, microalbuminuric and macroalbuminuric with UACR less than 30 mg/g, from 30–300mg/g, and above 300mg/g respectively. ELISA (Exocell USA) was used for Nephritin estimation. Statistical evaluation was done.

Results: Nephritinuria was present in 70 (89.7%) out of 78 diabetic patients including 35 (81.4%) normoalbuminurics, 5 (6.4%) micro-albuminurics and all 5 of macroalbuminurics (p < 0.027). When associated with duration of diabetes, 21 (91.3%) positive with less than three years of diabetes, 24 (92.3%) with three to seven years and 25 (86.2%) in more than seven years (p < 0.039). Nephritin levels were found increasing from Normo-(0.86µg/ml) to Micro-(11.6µg/ml) to Macroalbuminuria group (47.6µg/ml),

compared to 0.15 µg/ml in comparison group.

Conclusions: The increase in nephritin levels from 0.86µg/ml in patients with normal albuminuria to 47.6µg/ml in patients with macroalbuminuria suggests that Nephritin precedes albumin in urine predicting early signs of kidney damage.

BIOINFORMATICS NAVIGATION PREDICTED STRUCTURE-FUNCTION DYNAMICS OF CALPAIN10 ISOFORMS

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Introduction and Objectives: Calpain10, a member of cysteine proteinase family, is present in eight different isoforms (a-h) exact mechanism through which it influences the insulin secretion and action is scanty.

Methods: We have applied bioinformatics approach to envisage its mechanism of action and possible involvement in diseases. National Center for Biotechnology Information Blast was used for humans' nucleotide and protein sequence alignments of the calpain10 isoforms a, c and g. Further, NCBI conserved domain tool and CDART were used for other species.

Results: Results of blast showed 100% nucleotide whereas 91% and 97% amino acid sequence identity among isoforms of Homo sapiens' calpain10 c and g with calpain10 a. There was 82% query coverage of calpain10 c and 17% of calpain10 g with calpain10 a. Conserved domain analyses of peptide sequences indicated domains IIa and IIb have significant homology with the catalytic domain of the cysteine protease superfamily in all three isoforms. The domain III and IV of isoform a and domain III of isoform c were found homologous with linker C2 like subdomain III of mu-calpain.

Conclusion: It is predicted that calpain10 a and c isoforms may have a role in developing diseases such as neurodegenerative, cardio-vascular, cataract, osteopenia and cancer. Whereas isoform g may exhibit protease activities having papa-in-like domain only.

TP53 PROTEIN OVEREXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA (OSCC): CORRELATION WITH HISTOLOGIC VARIABLES AND 5 YEARS SURVIVAL IN PAKISTANI PATIENTS

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Introduction and Objectives: Alteration of TP53 gene in oral squamous cell carcinoma (OSCC) is believed to be associated with reduced overall survival (OS), the 5 year prognosis in our study and the disease-free survival (DFS). The aim of this study is to determine whether TP53 protein over expression in OSCC is a prognostic indicator of survival in Pakistani cancer patients along with its correlation with risk factors including smoking, chewing habits, histological variables like grade and stage of the tumor in a high risk population.

Methods: A total of 140 patients of OSCC were part of our study. TP53 protein over expression was investigated by means of immunohistochemistry.

Results Overexpression of p53 protein was observed in 75 patients (54%) using a threshold of 10% stained tumor nuclei. Patients with p53 negative tumors had improved OS when compared with patients with p53 positive tumors. This difference was statistically significant (p=0.036) in univariate Cox regression analysis however, it lost its worth in the multivariate analysis.

Conclusion: This works supports that patients with p53 overexpression had a significantly poor overall survival compared to p53 negative patients. However, p53 overexpression was not associated with patient's disease free survival.

CARDIOVASCULAR EFFECTS OF AQUEOUS-METHANOLIC EXTRACT OF BERGENIA LIGULATA IN EXPERIMENTAL ANIMALS

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Introduction and Objectives: Prevalence of hypertension, a major risk factor for diseases like ischemic heart disease and cerebrovascular stroke, is on the rise. The aim of this study was to explore the cardiovascular effects of the aqueous-methanolic extract of *Bergenia Ligulata* rhizome (Bl.Cr), to provide pharmacological basis for its medicinal use in hypertension.

Methods: Aortae from Sprague-Dawley rats and atria from guinea-pigs were placed in 5 ml and 15 ml isolated tissue bath assemblies respectively, which were maintained at 37°C with constant carbogen supply, filled with physiological salt solution (Kreb's solution) and connected to a force transducer and Power-Lab attached with a computer.

Results: Bl.Cr, in isolated guinea-pig atria, equally inhibited force and rate of spontaneous atrial contractions. When tested on phenylephrine (PE, 1 µM) and K⁺ (80 mM)-induced vasoconstriction, Bl.Cr caused a concentration-dependent relaxation and also caused a suppression of PE (1 µM) control peaks in Ca²⁺-free medium.

Conclusions: These data indicate that Bl.Cr exhibits cardio-suppressant and vaso-dilatory properties. The vasodilator effect of the plant extract is mediated through inhibition of Ca⁺⁺ influx via membranous Ca⁺⁺ channels as well as Ca⁺⁺ release from intracellular stores. Further studies are required to elaborate the anti-hypertensive activity of the plant.

ASSOCIATION OF HEPATITIS G WITH LIVER DYSFUNCTION IN TREATMENT RESPONDERS HEPATITIS C PATIENTS

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Background: About 10 million Pakistani population is infected with Hepatitis C virus (HCV). The prevalence is even more pronounced among high risk population. Combination of standard interferon and ribavirin is still the first line therapy with sustained viral response (SVR) up to 40-50%. Pool of relapsers and non-responders is increasing in Pakistan and needs treatment with pegylated interferon plus ribavirin (peg IFN/RIB). The hepatitis G virus (HGV), a recently identified member of the Flaviviridae family, can cause chronic infection in man

Methods: 250 treatment responders Hepatitis C patients (undetectable HCV RNA in the serum after 24 weeks of post treatment follow up) were recruited from Jinnah Post Medical College and Ziauddin Hospital.

Liver function tests of all patients were assessed by automatic analyzer kits. HGV RNA was investigated in serum samples by reverse transcription and polymerase chain reaction amplification of the 5' non-coding region of HCV and hybridisation to a specific probe. The liver function tests of HGV RNA sero-positive and sero-negative patients were compared.

Results: A total of 250 treatment responder hepatitis C patients were evaluated with a mean age 44± 5.55. Males constituted 38.4 % (N=96) while female constituted 61.6% (N=154) of our study group. Mean total bilirubin, direct bilirubin and indirect bilirubin was 0.772 ± 0.19 mg/dl, 0.308 ± 0.99 mg/dl and 0.496 ± 0.55 mg/dl respectively. Mean ALT was 81.97 ± 38.03 units/L and total protein was g/dl 5.25 ± 0.44. Statically significant difference of mean LFTs was found between hepatitis G positive and negative patients with p-value = 0.001. To find out association of LFTs with hepatitis G positive patients, chi-square was used. Total bilirubin, direct bilirubin, indirect bilirubin, AST, GGT, and APTT are found to be associated with hepatitis G with p-value of 0.001. By Log Regression we found significant and positive association of Hepatitis G with Total bilirubin, direct bilirubin, indirect bilirubin, GGT and APTT (p-value = 0.001 at 95% CI).

Conclusions: Liver dysfunction in treatment responder

hepatitis C patients is found to be associated with novel Hepatitis G virus.

COMBINATION THERAPY OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AND ANTI-OXIDANTS: AN EFFECTIVE REGIMEN FOR INFLAMMATORY DISORDERS?

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Introduction and Objectives: Non-steroidal anti-inflammatory drugs are of therapeutic value for inflammatory disorders. Reactive Oxygen Species may initiate and/or aggravate inflammation. Information regarding the interaction of vitamin E with NSAIDs in inflammation is not well documented and is controversial.

Methods: This study was conducted to determine the effect of aspirin with vitamin E supplementation in inflammation, and to explore the possible interactions of vitamin E with one drug from each sub group of NSAIDs with respect to their anti-inflammatory activity using animal model of inflammation, DNA degradation and lipid peroxidation assays.

Results: Our results showed that vitamin E caused 10% inhibition in edema at 400 mg/kg dose while aspirin elicited complete inhibition in edema at 200 mg/kg dose. Co-administration of different doses of aspirin with vitamin E showed a dose-dependent increase in edema inhibition. At 100 mg/kg aspirin showed ~50% inhibition in edema. While the same dose of aspirin with vitamin E supplementation (200 mg/kg) showed a significant increase (~25%) in its anti-inflammatory effect. Similar results were obtained with DNA degradation and lipid peroxidation assays.

Conclusions: In conclusion, aspirin, diclofenac sodium, and celecoxib, with vitamin E supplementation, showed significant synergistic effects that could be used as an effective preventive and therapeutic regimen for inflammatory diseases.

CLINICAL EFFICIENCY AND COST EFFECTIVENESS OF MACROPROLACTIN SCREENING IN HYPERPROLACTINEMIC PATIENTS

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Introduction and Objectives: Macroprolactin (MaPRL) is a biologically inactive compound which leads to falsely elevated prolactin levels. It is recommended that all sera with increased total prolactin concentrations be sub fractionated by PEG precipitation to measure the bioactive monomeric prolactin concentration to prevent misdiagnosis and unnecessary investigations. We aim to determine clinical efficiency and cost effectiveness of PEG screening of hyperprolactinemic sera.

Methods: In this Retrospective cross sectional study patients with high total prolactin levels were screened by PEG precipitation. Relevant diagnosis of Macroprolactinemia and True Hyperprolactinemia was made based on their absolute PEG treated monomeric prolactin level. They were then contacted on phone and a detailed history of their clinical symptoms along with their radiological workups was inquired.

Results: Frequency of macroprolactin was 60.7% seen in 145 patients and true hyperprolactinemia was observed in 94 (39.3%) patients. More asymptomatic patients were reported in the macroprolactin as compared to true hyperprolactinemic group (p = < 0.05). 37 (39.4%) patients with true hyperprolactinemia had further radiological investigations done where-as only 8 (5.5%) of the patients with macroprolactin had undergone further radiological workups. The total use of resources in the true hyperprolactinemic group was significantly higher, 943000 PKR (10031 PKR per person) vs 418450 PKR (2884 PKR per person) (p = < 0.05).

RETINAL NERVE FIBER LAYER THICKNESS IN A SUBSET OF KARACHI (PAKISTAN) POPULATION

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Introduction and Objectives: To provide the normal range of retinal nerve fiber layer (RNFL) thickness in a subset of Karachi population by Spectralis OCT and to evaluate the effects of age and gender on it.

Methods: 300 eyes from 150 healthy subjects aged 40 years and above with no ocular pathologies were examined using standard protocols by a single examiner. Subjects with history of diabetic or hypertensive retinopathy, raised intraocular pressure (> 21mmHg) and previous intraocular or laser surgery were excluded from the study. The mean retinal nerve fiber layer thickness was calculated and was correlated with age and gender difference.

Results: The mean global retinal nerve fiber layer thickness was found to be $99.02 \pm 9.08 \mu\text{m}$ in our set of population. Out of four quadrants the maximum RNFL thickness was found in inferior quadrant ($126.45 \pm 16.23 \mu\text{m}$) followed by the thickness of $121.50 \pm 15.03 \mu\text{m}$ in superior quadrant, $70 \pm 14 \mu\text{m}$ in nasal quadrant and $68.90 \pm 13.10 \mu\text{m}$ in temporal quadrant. We found strong negative correlation of RNFL thickness with age ($P < 0.001$) and not significant relation with gender ($P = 0.8$).

Conclusions: Keeping in mind the variations in RNFL thickness with ethnic differences, this study provides the normal values of RNFL thickness according to our set of population. It is concluded that RNFL thickness decreases significantly with increasing age but gender had no significant effect on it.

PREVALENCE OF METABOLIC SYNDROME IN ADOLESCENTS /FIRST YEAR MEDICAL STUDENTS OF A PUBLIC SECTOR MEDICAL COLLEGE IN QUETTA, PAKISTAN

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Introduction and Objectives: Metabolic syndrome is a disorder of energy utilization and storage. According to the International Diabetes Federation, metabolic syndrome in adolescents more than 16 years is defined as “the presence in an individual of central obesity plus any two of the following four risk factors: hypertriglyceridemia, hypertension, low high density lipoprotein and high fasting glucose levels.

Reports suggest that around 25% of the world’s adult population suffers from metabolic syndrome. People with metabolic syndrome are twice as likely to die and have three times higher chances of heart attack or stroke.

The study is designed to determine the prevalence of metabolic syndrome in first year medical students between 17-19 years, of a public sector medical college in Quetta.

Methods: A Cross-sectional study was conducted on all the healthy first year medical students, ages from 17-19 years studying in a public sector medical college in Quetta, Pakistan. Demographic data were assessed by using self-administered questionnaire. Height, weight and waist circumference were measured along with fasting blood sugar, high density lipoprotein and triglycerides by using Automated Biochemistry Analyzer. Analysis was done on SPSS 20. Descriptive statistics was used to present the data and logistic regression was applied to see the relationship between the study variables ($P < 0.05$).

Results: A total of 225 were included in the study, out of which ($n=127$, 56.4%) were females. Mean age was 18.79 ± 0.38 years. Prevalence of metabolic syndrome was found to be 14.2% in our study sample. Increased waist circumference, fasting blood sugar, triglycerides were associated with an increased likelihood of exhibiting metabolic syndrome. Male gender and high HDL were

found to be protective against metabolic syndrome.

Conclusion: Prevalence of metabolic syndrome is increasing in our adolescent population which demands screening from childhood and early adolescence. Early screening, identification and lifestyle interventions will decrease the morbidity from chronic illnesses like Coronary artery disease and Diabetes mellitus.

ASSOCIATION OF VITAMIN D WITH OUTCOME AFTER INTRA CYTOPLASMIC SPERM INJECTION

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Introduction and Objectives: To observe effects of vitamin D levels on pregnancy outcome after intra cytoplasmic sperm injection (ICSI).

Methods: It was a cross sectional study conducted in Australian Concept Infertility Medical Centre from July 2011 to August 2014. Estimation of 25 hydroxy cholecalciferol (25-OHD) of consented females (252) was done before treatment protocol for ICSI. Results of β hCG performed 14 days after embryo transfer categorized groups; Pregnant with β hCG more than 25 IU/mL and rest included in non-pregnant group. Both groups were compared by independent sample t-test and Pearson Chi Square test. Binary Logistic Regression Analysis was used to estimate odds ratio of pregnancy outcome with its predictors.

Results: The mean value of 25-OHD, number of oocytes, fertilized oocytes and endometrial thickness was significantly higher in pregnant women. A significant positive association of 25-OHD with clinical pregnancy and thickness of endometrium was observed. After adjustment with female age and BMI positive association of vitamin D with endometrial thickness was observed.

Conclusion: Deficiency of (25-OH) in females hinders the accomplishment of optimal endometrial thickness required for implantation of embryo after ICSI. The improvement in vitamin D status can thus improve success results in assisted reproductive clinics.

COMPARISON CLINICAL OUTCOME AND COST-EFFECTIVENESS OF TEGAFUR AND CAPECITABINE FOR THE TREATMENT OF METASTATIC COLORECTAL CANCER

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Introduction and Objectives: The development of numerous oral chemotherapy agents has led to a new paradigm in management of cancer. Treatment modalities with cost effective approach and better clinical outcomes can be proved to be an effective strategy to reduce disease burden.

To evaluate the clinical and cost effectiveness of oral chemotherapeutic agents Capecitabine and Tegafur as first-line treatments for patients with metastatic colorectal cancer.

Methods: The analysis involved 121 patients of metastatic colonic carcinoma admitted at Almehrab Tibi Amdad Hospice care, Karachi between September 2013 to March 2015. The patients selected to enrolled in present study were aged ≥ 18 years, both sex and histologically diagnosed cases of colorectal cancer (stage 3 with primary surgical resection). While those with history of hypersensitivity to Tegafur, fluoropyrimidines, capecitabine, or any other ingredients of this product, Inadequate hematopoietic function WBC 4,000/mm³; ANC 2,000/mm³; Platelet 100,000/mm³, Inadequate organ function, CNS metastasis, life expectancy less than 3 months and those who were not willing to participate were excluded. All subjects fulfilling the eligibility criteria were randomly assigned to two groups, one group received oral capecitabine (1250 mg/m² twice daily for 14 days followed by one week gap) and other received Capsule Tegofer 500 mg daily for 28 days followed by one week gap. All the patients were followed and

response was observed after 3 months, 6 months and at the end of year.

Results: Patients receiving Tegafer showed significantly less incidence of alopecia, diarrhea and stomatitis ($P < 0.05$) while incidence of hand-foot syndrome and grade 3/4 hyperbilirubinemia were found to be significantly more as compared to capecitabine ($P < 0.0001$). Cost of treatment per patient and per cycle using oral Tegafer was less than that using capecitabine.

Conclusion: Tegafer showed overall improved response rates, cost effectiveness and has lesser side effects in comparison with Capecitabine.

RADIOLOGICAL STUDY OF THE MEAN AGE OF FUSION OF MEDIAL END OF THE CLAVICLE AS A PARAMETER OF AGE

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Introduction and Objectives: Estimation of the age of a person is a problem that a forensic expert confronts in both living and dead subjects. 21 years of age is of immense medico-legal importance, as it entitles a person to be employed as a gazetted officer, getting driving license for all types of vehicles, participation in various sports events and is a mandatory parameter for identification in dead. This study gives us an authentic criterion for assessing 21 years age, by using closure of ossification centers at medial clavicular epiphysis by applying radiological method for subjects living in Karachi.

Methods: The main objective of this study is to determine the mean age of fusion of medial end of clavicle by radiological method, to get a perfect estimator of 21 years of age.

Results: The mean age of fusion of medial end of the clavicle was found to be 21 ± 1.43 years. Significant difference was observed in male to female ratio 21.14 ± 1.41 versus 20.65 ± 1.94 (P value < 0.05). Similarly, statistically significant difference was observed between lower class to middle class and higher class of all society. No difference was observed between the various ethnic groups.

Conclusion: Socio-economic factors such as diet and nutrition directly affects bone growth and hence bone age. The results of fusion of the medial end of the clavicle are not affected by ethnicity. More studies should be conducted in various parts of country to make a natural standard in setting up uniform criteria for assessing age at or above 21 years.

CENTRATHERUM ANTHELMINTICUM (KALIZERI) MINIMIZE THE RISK OF CHEMICALLY-INDUCED HEPATOTOXICITY IN RATS

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Introduction and Objectives: Carbon tetrachloride (CCl₄) is a cleaning agent used in industry. Its vapours are injurious for many body tissues especially liver. The study was designed to evaluate the hepatoprotective effect of ethanolic seeds extract (ESEt) of *Centratherrum anthelminticum* (kali zeri) in CCl₄-induced hepatotoxic rats.

Methods: Rats were made hepatotoxic by intraperitoneal injection of CCl₄ (3 ml/kg) on 3rd and 5th day of trial and divided into hepatotoxic control (distilled water 1 ml/kg), positive control (silymarin 100mg/kg), and test (ESEt 800 mg/kg) groups. Normal control rats were also run. After 24 hours of last dose of CCl₄, body weights (BW) of all rats were recorded, sacrificed them to collect blood and serum to analyze liver-specific parameters. In addition, livers were dissected out carefully to estimate lipid peroxidation (LPO).

Results: ESEt (600 mg) not only significantly decreased the percent reduction in BW of test rats but also improved the levels of alanine transferase (ALT), aspartate transferase (AST), alkaline

phosphates (ALP), total bilirubin, total protein and albumin. Beside these, percent inhibition of LPO was also increased.

Conclusion: The ESEt of *C. anthelminticum* was found as a hepatoprotective and antioxidant agent that could be used for the preparation of medicine in future.

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

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Introduction: Gastrointestinal malignancies are the global oncological problems. It is therefore imperative to investigate the spectrum of this pathology in Pakistan.

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

Methods: The study was conducted during 2009 to 2014. All the cases of preneoplastic and neoplastic lesions of intestine received during the period of 6 years were reviewed. The data was subjected to statistical analysis using SPSS version 22.

Results: A total of 64 cases were diagnosed as premalignant lesions of intestine consisting of ulcerative colitis (19/29.7%), adenomatous polyp (17/26.6%), dysplasia (14/21.9%) and adenoma (14/21.9%).

About 478 cases were diagnosed as malignant lesions of intestine as:

- i. Adenocarcinoma grade I (65/13.6%), grade II (283/52.2%), grade III (95/19.9%)
- ii. Squamous cell carcinoma grade I (2/0.4%), grade II (7/1.5%), grade III (3/0.8%)
- iii. Metastatic adenocarcinoma (19/4.0%)
- iv. Neuroendocrine (4/0.8%)

Conclusion: Our study showed ulcerative colitis as the commonest premalignant lesion and grade II adenocarcinoma the most common malignancy of intestine.

COMPARISON OF ANTIBACTERIAL ACTIVITY OF PUNICA GRANATUM AND SYZYIUM CUMINI LEAVE EXTRACTS

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Introduction and Objectives: Plants are the richest source of phytochemicals, not only protecting the plants itself but also benefits the mankind. *Punica granatum* commonly known as pomegranate and *Syzyium cumini* known as Jamun are well known for its phenolic compounds having antioxidant potential. They are used in traditional medicine for the treatment of diabetes, diarrheal diseases, antiparasitic and antibacterial infections.

Methods: The aim of the study was to identify the antimicrobial potential of two leaf extracts of *Punica granatum* and *Syzyium cumini*. Fresh leaves were washed and ground with distilled water. The extract was obtained which was filtered by Whatman #1 filter for the removal of particulates. The antimicrobial activity was performed by agar well diffusion method against *E. coli*, *Bacillus subtilis*, *Klebsiella oxytoca*, *Salmonella typhi*, *Micrococcus leutus*, *Proteus mirabilis*, *Staph aureus* and *Candida albicans*. PBS was taken as negative while streptomycin as positive control.

Results: It was found that the extract of *Punica granatum* was inhibitory to all microorganisms. The highest zone of inhibition was observed against *Proteus mirabilis* and *E. coli* (25mm). Leaf extract of *Syzyium cumini* do not show any antimicrobial activity.

Conclusions: Pomegranate leaf extract can be effective against GIT infections. Jamun, as reported earlier, is well known to control diabetes cannot be used to control infections.

IDENTIFICATION, DIFFERENTIATION AND SENSITIVITY PATTERN OF HAEMOPHILUS INFLUENZAE AND HAEMOPHILUS PARAINFLUENZAE

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Introduction and Objectives: H.influenzae is a pleomorphic Gram-negative coccobacillus that is isolated predominantly from the respiratory tract and this opportunistic species may cause systemic infections such as epiglottitis, meningitis, bacteremia/sepsis, bronchitis and chronic obstructive pulmonary disease and otitis in young children. H. parainfluenzae, in contrast, is a saprophyte that colonizes the upper respiratory tract but it hardly causes respiratory tract infections and only occasionally infectious endocarditis. But many strains are now becoming resistant to beta lactam antibiotics. We aim to test the sensitivity pattern of Haemophilus influenzae and Haemophilus parainfluenzae.

Methods: Total 45 sputum samples were analyzed collected from different hospitalized patients. Specimens were inoculated on blood agar plates and incubated in 5-% CO₂ at 37°C for 24-72 hours. Gram stain were perform for direct examination and differentiation was done on the basis of availability of hemin(X) and NAD (V) factor, oxidase and slide agglutination test. Antibiogram was done by CLSI method. Statistical analysis was also performed using SPSS version 17.

Results: H.parainfluenzae showed such sensitivity pattern AMP75%, AMC80%, CRO80%, SXT42%, C92%, CIP/OFX84% while H. influenzae showed 100% sensitivity towards AMP, AMC, CRO, SXT, C and 92% sensitivity against CIP.

Conclusions: Proper diagnosis and selective use of antibiotics can minimize the resistivity of these organisms.

ASSOCIATION BETWEEN INTERLEUKIN 6 GENE POLYMORPHISM AND HUMAN PAPILLOMA VIRUS INFECTION IN ORAL SQUAMOUS CELL CARCINOMA PATIENTS

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Introduction and Objectives: Human papilloma virus (HPV) and IL 6 gene polymorphism are proven independent risk factors for development of Oral Squamous Cell Carcinoma. The objectives of this study were to find out frequency of IL6 gene polymorphism and HPV and to evaluate any association between HPV and IL6 gene polymorphism in OSCC patients and its effect on disease prognosis.

Methods: In this cross-sectional study 140 OSCC patients (104 males and 36 females), aged 18yrs and above were selected. Detailed questionnaire was filled followed by sample collection. After DNA extraction PCR analysis for HPV and RFLP for IL6 gene polymorphism was carried out.

Results: Mean age of the patients was 43.5±11.84 years (range 31-40 years). Majority (n=45; 32.1%) belonged to Urdu speaking ethnic group and were habitual eaters of Pan (n=87; 62.1%) and Gutka (n=82; 58.6%). The most common site of OSCC was buccal mucosa (61.4%). Most of the patients presented with grade II (55%) and late stages (stage III & IV) (55.7%) of OSCC. Out of 140 samples, 12 (8.6%) tested positive for HPV gene with the following pattern of IL 6 gene polymorphism; GG (46.4%), GC (39.3%), CC (14.3%). A significant association was observed between stages III & IV of OSCC and IL6 genotypes GC (P=0.001) and CC (P=0.002). Also strong positive association (P=0.003) was found between HPV and IL 6 (P=0.007) CC homozygote genotype.

Conclusions: This study finds an association between HPV and IL6 gene polymorphism in late stage OSCC patients suggesting rapid and aggressive progress of oral carcinogenesis.

COMPARISON OF VIRULENCE FACTORS OF ISOLATED CANDIDA SPP FROM LOWER RESPIRATORY TRACT INFECTIONS IN HOSPITALIZED PATIENTS

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Introduction and Objectives: Candida a diverse group of fungus has bacterial and fungal properties and 80% commensals of normal human body i.e C. albicans and C. tropicalis are involved in various diseases like oral thrush, vulvovaginal candidiasis etc. when they get opportunity and they now are considered as the third most pathogenic strain causing various diseases in human. The objective is to compare the virulence properties of different Candida spp.

Methods: 126 sputum samples were collected from May to August 2015 from hospitalized patients. Samples were inoculated on SDA. Identification was processed by gram staining, germ tube test, Chlamyospore detection and CHROM agar. Phospholipase, proteinase and hemolytic activity were deliberated in them. Statistical analysis was also performed using SPSS version 17.

Results: 51% males and 48% female were found to be infected with Candida spp. The most prevalent organism was C. albicans (87.5%) followed by C. glabrata (8.56%) and C. tropicalis (3.08%). Phospholipase activity (45.9%), proteinase (30.1%) and haemolysin activity (19.9%) was shown by C.albicans. Phospholipase and proteinase activity was also observed in C.tropicalis but not in C. glabrata.

Conclusions: Present study indicate that C.albicans being a member of normal flora pose a major threat in immune-compromised patients and majors should be enforced to prevent infections with Candida.

STRUCTURAL INVESTIGATION OF NEISSERIA MENINGITIDIS PATHOGENIC FACTORS; A KEY STEP TOWARDS DRUG DESIGNING

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Introduction and Objectives: The Gram negative human pathogenic bacterium Neisseria meningitidis is responsible for causing meningitidis and septicemia worldwide. A substantive question in bioinformatics analysis of bacterial genome is to ascribe a three dimensional structure as well as a biologic role to all the coding regions. Here we performed structural bioinformatics analyses of important Neisseria meningitidis pathogenic factors involved in protein biosynthesis including methionyl-tR-NA synthetase, 16SRNA methyltransferase, translation elongation factor-Tu and putative RNA methylase.

Methods: Homology modeling of these important drug targets was carried out with better templates by MODELLER software and evaluated by Prosa and Procheck standalone softwares.

Results: The study provided detailed structures of important proteins required for the pathogenesis of this organism.

Conclusions: In future, these investigations will help in receptor-based drug designing against meningococcal infections.

EVALUATION OF ANTIMICROBIAL ACTIVITY OF ALOE VERA

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Introduction and Objectives: Aloe Vera is a cactus, belongs to the family Liliaceae, and is well known for its use as traditional medicine. It is used for gastrointestinal disorders, stimulates body's immune response, for treatment of burns, eczema, psoriasis, to heal skin infections. Aloe Vera is known to hold therapeutic properties due to bioactive components present in it for both infectious and non-infectious diseases.

Methods: Antimicrobial activity of Aloe vera was investigated against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Micrococcus, Klebsiella pneumonia, Proteus, Staphylococcus aureus, Salmonella typhi and Candida albicans. The organisms were isolated from clinical samples and identified by conventional

methods. Susceptibility test was carried out by agar well diffusion method. Aloe vera used as methanolic extract and pure gel. All experiments were performed in triplicate.

Results: Zone of inhibition was observed in all forms of Aloe vera. The maximum zone of inhibition was found in E.coli and followed by Pseudomonas aeruginosa, Salmonella typhi, S aureus and Bacillus subtilis showed least zone of inhibition. It was also found that inhibitory affect against Candida albicans was also maximum.

Conclusions: The result of this study would tend to give credence to the use of Aloe Vera for the formulation of certain compounds that would coin new, effective and more potent antimicrobial drugs to combat pathogenic microorganisms.

BIOINFORMATICS ANALYSIS OF HISTONE-LYSINE N-METHYLTRANSFERASE - SETD1A GENE IN ASSOCIATION WITH SCHIZOPHRENIA

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Bioinformatics methodologies have made it possible to profile the global composition of tissue or organelle at specific time point or under particular developmental or disease state. A genetic variant of SETD1A has yielded significant genome-wide association with schizophrenia, suggesting that this SETD1A plays a key role in its etiology. The SETD1A gene plays a part in chromatin modification - an important cellular process that reduces the size of DNA so it can fit inside a cell and regulate gene expression. It encodes a catalytic subunit of an enzyme that adds methyl groups to histone proteins - genes for transcription.

The present study is based on computational analysis of SETD1A gene exploring molecular networks of interacting targets by using advanced bioinformatics tools such as, STRING's, KEGG, Reactome and BioGrid databases that may provide insights into the biological processes underlying schizophrenia.

FREQUENCY OF HEPATITIS B AND C IN DIFFERENT AREA OF CATCHMENT CENTER IN GADAP TOWN, KARACHI, PAKISTAN

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Introduction and Objectives: Frequency of Hepatitis B Surface Antigen (HBs Ag) and Hepatitis C Virus Antibodies (anti-HCV) among population of different catchment center in Gadap area with their low social economic status. We aim to study the frequency of Hepatitis B Surface Antigen (HBs Ag) and Hepatitis C Virus Antibodies (Anti-HCV) in different catchment center of Gadap town in 12 kilometer from Baqai Medical University. The design of study will be cross sectional descriptive study. It was held in the Pathological and Molecular Laboratories, Karachi, during the period October, 2011 to May 2012.

Methods: A total of 496 samples were collected. Serum was tested for Hepatitis B surface antigen and for Hepatitis C virus antibodies by ELISA. The results were subjected to chisquare analysis for determination of statistical difference between the values among different categories.

Results: Among 496 patients 91 (18.3%) were positive for HB s Ag and 171 (34.4%) were positive for Anti-HCV. The frequency of HB s Ag was seen too low as compared to anti-HCV.

Conclusions: High frequency of HCV infection needs implementation of strict screening policy for donors and public awareness campaigns about preventive measures to reduce the spread of this infection as well as other transfusion transmissible infections.

INNOVATIVE APPROACHES IN METABOLOMICS FOR

UNDERSTANDING DRUG RESISTANCE IN BREAST CANCER

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Introduction and Objectives: Breast cancer is one of the leading cause of death worldwide. In Pakistan, prevalence of this ailment is highest amongst all types of cancer i.e. 38.5%. Major clinical setback is drug resistance in breast cancer. Metabolomics is an emerging field that utilizes information of cellular biochemistry for the early detection, diagnosis and establishment of predictive biomarkers of breast cancer. This review highlights potential metabolomic applications to pharmacology and clinical pharmacology.

Methods: The methodology is based on inclusion exclusion criteria. Literature survey, and questionnaire were included while clinical trial was excluded. This report provides a review of 12 articles out of few were excluded.

Results: According to the survey the average response rate of a cancer drug is the lowest at 21%, suggesting that 74% of patients with cancer are over-dosed. While according to an international study, 40%–50% of breast tumors will display acquired resistance.

Conclusions: When specific therapies are chosen on the basis of a patient's metabolomics profile, it will give rise to customized medicine and personalized tailored treatment. Using high-throughput information using metabolomics to clinical diagnosis and treatment can help accelerate the patient safety, quality of life and survival rate by identifying pathways involved in drug resistance.

ABERRANT PROTEIN S-NITROSYLATION IN HYPERTENSIVE AND DIABETIC-HYPERTENSIVE PATIENTS: NEW PERSPECTIVE

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Hypertension and diabetes are the risk factor of morbidity and mortality. The prevalence of hypertension is expected to increase in the upcoming years. The incidence of diabetes is two fold higher in patient with hypertension. The co-existence of diabetes and hypertension causes impaired renal function, the development of diabetic retinopathy, cardiac problems, and the development of cerebral diseases. Besides these disorders hypertension and diabetes are involved in many other devastating disorders. Protein S-nitrosylation conveys a large part of the ubiquitous effect of nitric oxide on cellular signal transduction, accumulating evidence indicates important roles for S-nitrosylation both in normal physiology and in a broad spectrum of human diseases. Dysregulated S-nitrosylation has been implicated as a cause or consequence of a broad range of diseases, including asthma, cystic fibrosis, Parkinson disease, heart failure, and stroke. The role of nitrosylases and denitrosylases in governing levels of S-nitrosylation under both physiological and pathophysiological conditions is increasingly appreciated. The purpose of study is to identify s-nitrosylation in hypertensive and diabetic hypertensive serum samples. We take serum sample of hypertensive subjects (n=10), diabetic hypertensive subjects (n=10) and normotensive subjects (n=10). Total protein was quantified using Bradford assay. SDS PAGE was done after dilution of sample followed by western blotting. The results we obtained from the above mentioned methods showed that s-nitrosylation is decreased in hypertension and slightly increased in diabetic hypertension.

STRUCTURAL BIOINFORMATICS ANALYSIS OF TRANSCRIPTION FACTORS INVOLVED IN NEISSERIA MENINGITIDIS PATHOGENESIS; A LEAP TOWARDS ANTIMENINGOCOCCAL DRUG DISCOVERY

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Introduction and Objectives: *Neisseria meningitidis* is a virulent pathogen causing meningitis as well as life-threatening septicaemia throughout the world. Unfortunately, any vaccine or drug has not yet been developed against *N.meningitidis* serogroup B. Functional genomics strategies have been adapted to study the growth and pathogenesis of *Neisseria meningitidis* in which a library of 2850 insertional mutants was analysed and 73 genes were identified in *N.meningitidis* genome responsible for causing disease.

Methods: We have selected the proteins (gene products) i.e., Dead box RNA-Helicase, Polyribonucleotide nucleotidyl-transferase PNPase and Ribonuclease-III involved in transcription for their detailed structural analyses. These proteins are also involved in RNA-degradosome assembly.

Results: Different bioinformatics strategies were applied and the homology models of these proteins were built using protein structure-modelling program MODELLER and the models were evaluated using PROSA and PROCHECK software, as well as active sites are also predicted.

Conclusions: Since these proteins are potent drug targets, therefore their structural identification will prove to be a breakthrough in drug discovery. We hope that our study will grease the wheels for drug designing against lethal meningococcal disease.

ROLE OF RAUWOLFIA SERPENTINA EXTRACT ON THE MODULATION OF IMMOBILIZATION STRESS-INDUCED BEHAVIORAL DEFICITS AND ADAPTATION TO STRESS IN RATS

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Introduction: Stress is the major precipitating factor in the onset of depression, anorexia nervosa, diabetes and obesity. The goal of present study is to monitor the behavioral and biochemical effects of post stress administration of *Rauwolfia serpentina* extract on adaptation to immobilization stress in rats.

Methods: The plant extract (30 mg/kg) was orally administered after termination of immobilization stress daily (2 hours) for 5 days to monitor any change in behavioral activities. Effects of *Rauwolfia serpentina* extract on immobilization stress induced deficits of food intake and body weight were determined for an understanding of the relationship between stress tolerance and behavioral changes. We also tested effects of *Rauwolfia serpentina* extract on endogenous leptin and glucose levels in unstressed and stressed animals to explore the possible role of HPA axis in the modulation of stress-induced behavioral deficits and adaptation to stress.

Results: The present study showed that *Rauwolfia serpentina* extract can blunt stress-induced anorexigenic as well as anxiogenic-like effects. Moreover, *Rauwolfia serpentina* extract reversed adverse effects of stress and facilitate adaptation to an uncontrollable stressor by reducing stress perception.

Conclusion: The present study shows that immobilization stress induced decreases in food intake, body weight as well as behavioral deficits were reversed by *Rauwolfia serpentina* extract suggesting anxiolytic like profile of drug. This effect of *Rauwolfia serpentina* extract can be explained in terms of additive effects of stress on serotonin (5-hydroxytryptamine; 5-HT) neurotransmission particularly via postsynaptic 5HT_{2C} as well as 5HT_{1A} receptors. It is therefore suggested that post stress administration of *Rauwolfia serpentina* extract provides an innovative approach for the treatment of stress related disorders.

ALTERATION IN SALIVARY PARAMETERS LEAD TO ORAL LESIONS AMONG CHEWABLE TOBACCO USERS

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Introduction: Salivary flow and composition alters under deleterious chemical irritants present in chewable tobacco. Apart from inflammation and deterioration in health of oral mucosa it can also affect the major and minor salivary glands and cause a decline in salivary flow rate. The objective of the study was to find out alteration in salivary parameters that lead to oral lesions among chewable tobacco users

Methods: A total of 354 healthy male subjects, consuming any form of chewable tobacco product, belonging to low socio-economic areas of Karachi were selected for this cross sectional study. A questionnaire was used to collect demographic data and details of chewing habits (using since, pack/day, duration of exposure etc.). Resting saliva of every subject was collected for 5min and RSFR was expressed in ml/min. Salivary pH was determined by using pH strips (pH 0-14). Oral examination was done for the presence or absence of oral lesions. Data was analyzed on SPSS version 20.

Results: Out of the 354 subjects included, 27.4% consumed gutka, 24.3% niswar, 24.3% paan and 24% were multiple users. Mean Resting Salivary Flow Rate (RSFR) was 0.52±0.34 ml/min, pH 6.58±0.78 and 27.1% (n=96) had oral lesions. Highest frequency of oral lesions was found among subjects who had hyposalivation (40%) and those having acidic pH (40%). A significant decrease in RSFR and pH and increase in frequency of oral lesions is observed with increased duration of exposure, duration of usage and increased number of tobacco packs consumed per day.

Conclusion: Increased frequency and recency of chewable tobacco use leads to decrease in RSFR and pH and hence increase in frequency of oral lesions.

SPEECH ASSESSMENT THROUGH THETRI-POSITION ARTICULATION ANALYSIS FOR BILINGUALS (TAAB)

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Introduction: Bilingualism is regarded as a cognitive feat by today's linguists. The acquisition of more than a single language manifests the miracle of the human brain in terms of meta-linguistic (meta phonological) skills, as it stores the form and content of multiple languages and exhibits their use in socially and linguistically appropriate contexts.

Methods: Pakistan, like most Asian countries has a predominantly bilingual fabric. Little children, regardless of their socioeconomic status are most often exposed to their native language (L1) and to a second language (L2) within the critical period (0-3years), leading to simultaneous bilingualism. When they are exposed to L2 after the acquisition of L1 we call it sequential bilingualism. Some children may have L3 in their linguistic repertoire which may be the additional language such as the language of instruction at school. The development of articulation and phonological skills in children usually reaches maturation around the chronological age of 6 years. Children pick up several sounds of their language and perfect them gradually using approximations that sound closer and closer to the target sound, acquiring all sounds with the culmination of early childhood.

Results: Children may present with unclear speech, in the absence of an organic etiology (dyslalia or phonological disorders) or because of a known organic cause such as cleft lip/palate, malocclusions, hearing loss, dyspraxia or dysarthria, must be assessed for their ability to acquire phonemes of all the languages in their repertoire, because the brain stores auditory images of all sounds and uses them appropriately when needed.

Conclusions: The Tri-position Articulation Analysis for Bilinguals is a tool for testing the articulation and phonological skills of bilingual children aged 0–6 years, that uses culturally and linguistically appropriate picture stimuli for children exposed to Urdu, Sindhi, Punjabi, Pushtu, Hindi and English. The test assesses spontaneous elicitation of the sounds in the child's lexicon, along with stimulability and also assesses the function of the speech mechanism for non-speech functions.

STABILITY STUDIES OF METFORMIN HYDROCHLORIDE, AMLODIPINE BESYLATE AND CIPROFLOXACIN HYDROCHLORIDE TABLETS UNDER SPECIFIED CONDITION OF TEMPERATURE AND RELATIVE HUMIDITY OF PAKISTAN

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A pharmaceutical formulation ensures safety, protection, procurement and preservation of human health. Therefore, preservation of its physical and chemical stability throughout the period of its shelf life has always been the matter of prime concern for a pharmaceutical manufacturer. A good formulation is one that when packaged in a suitable container, (which provides maximum protection to its contents), confirms the absolute delivery of its active ingredient in its original form to its consumer. To determine the suitability of blister strips for packaging of Metformin HCl, Amlodipine besylate and Ciprofloxacin hydrochloride tablets, we maintained a stability profile for the changes in physical appearance and loss of potency of these medicines for a period of 2 shelf life years. Tablets were subjected to the specified conditions of 30°C and 65% RH for 24 months and samples were withdrawn at different time intervals. Different tests and assays were conducted on these samples showed that under these specified conditions the efficacy of these tablets in the blister strips retain for less than 2 shelf life years which is less than the expected shelf life of 3 years.

RETROSPECTIVE ANALYSIS OF MATERNAL MORTALITY AT ZIAUDDIN UNIVERSITY HOSPITALS, FROM 2012- 2014

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Introduction and Objective: To determine the frequency of maternal mortality and identify the causes with different age groups, parity and gestational ages in Ziauddin university hospitals in 4 campuses, from 2012- 2014.

Methods: A descriptive study conducted at Gynecology & Obstetrics units of Ziauddin university hospitals with retrospective review of validated records of hospital registers in 3 years from January 2012 to December 2014. Convenience sampling used to access the records. A self-structured proforma designed to collect data on variables (age, parity, gestational age, cause of death, condition at the time of admission, duration of stay in hospital before death, cause of delay).

Results: During 3 year period from January 2012 to December 2014 there were 32 maternal deaths. Total number of births were 14219 and 14184 were live births. Still births were 33. Eclampsia (21.8%) and haemorrhage (21.8%) were the leading cause of maternal mortality followed by those with puerperal sepsis (12.5%) and others. MMR (maternal mortality ratio) was 226 per 100000 live births.

Conclusion: Maternal mortality was high with leading causes as hemorrhage, eclampsia, and sepsis in ages of 20-30 years and parity 1-4. Mostly deaths were due to delay in getting the women to the health facility.

VITAMIN D DEFICIENCY IN PREGNANT WOMEN AND THEIR NEW BORN

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Introduction and Objectives: Vitamin D deficiency may affect the mother and its new born health. The aim is to detect the frequency of vitamin D deficiency in pregnant women and their newborn of Karachi at tertiary care hospitals, and correlating maternal vitamin D with cord blood.

Methods: This cross-sectional study was conducted on 50 women in labor presenting with a singleton term pregnancy at tertiary centers in Karachi. Data was recorded on a special proforma, maternal blood was taken before delivery and cord blood was taken at delivery. All blood samples were analyzed for 25-hydroxy vitamin D levels.

Results: The mean vitamin D levels were 24 ng/mL for the mothers and 20 ng/mL for the newborns. Vitamin D sufficiency was noted in 11 (22%), insufficiency in 16 (32%), and deficiency in 23 (46%) of the 50 mothers whereas sufficiency and deficiency, were noted in 6 (12%) and 44 (88%) of the newborns respectively. There was a positive correlation between the vitamin D levels in maternal and cord blood ($r=0.03$; $P=0.003$). Maternal vitamin D levels were significantly affected by sunlight exposure ($P=0.007$) and quality of diet ($P=0.01$).

Conclusion: Vitamin D deficiency is high among pregnant urban Pakistani women and their newborns. This public health problem needs urgent attention.

ARE YOU PREPARED TO CARE YOUR LOVED ONES: A CROSS SECTIONAL STUDY TO ASSESS THE LEVEL OF PREPAREDNESS AMONG CAREGIVERS OF CHRONICALLY ILL PATIENTS IN A TERTIARY CARE HOSPITAL AT KARACHI, PAKISTAN

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Introduction: Family caregivers are also known as informal caregiver. They provide all level of care at home to their loved ones most often equivalent to the professional care providers, such as providing physical care, giving medications recognizing early signs of severe conditions, changing dressings etc. While providing care to their loved ones most of them feel unprepared in terms of lack of knowledge and skills. This unpreparedness may result in the role strain and have may psychological consequences for the family. Nurses as a primary care provider could assess the caregiver preparedness and may educate them according to the patient's need to reduce the extra burden and the role strain.

Method: The self-administered instrument regarding the preparedness for care giving scale by Archbold (1990) has been utilized. The scale has eight items to identify the preparedness of caregiver in multiple domains. The responses are rated on five point scale with scores ranges from 0 (not at all prepared) to 4 (very well prepared). The higher score means caregiver feels more preparedness and vice versa. 50 caregivers of chronically ill patients were selected at a tertiary care hospital, which were caring for their loved ones for about six months or above. Written consent was obtained from the participants.

Result: 50 caregivers were recruited to fill the form from which 26 were female and 24 were male. All of the participants were between 18 years and 60 years of age. About 68% caregivers score between not too well prepared and somewhat well prepared. Whereas, only 32% score between somewhat prepare and pretty well prepared.

Conclusion: In conclusion, most of the caregivers score are somewhat prepared which is an alarm for nurses and all health care providers to work to reduce their role strain and provide sessions according to the need of the caregivers. Nurses could arrange teaching sessions which could be helpful for the caregivers to do their role effectively.

ABO AND RHESUS BLOOD GROUP DISTRIBUTION IN RESIDENTS OF KARACHI

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Introduction and Objective: Blood is an essential component for human beings and in disease states, it is requested to be transfused from healthy human donors. But it was continuously a life threatening procedure until the discovery of ABO blood groups. Today committee of League of Nations has recommended A, B, AB & O classification for general use. This study was aimed to determine the frequency of ABO and Rh blood groups. So that necessary measures should be taken to maintain the blood product inventory. Frequency of ABO and Rhesus blood group distribution in residents of Karachi.

Methods: A study was conducted at Dr. Ziauddin Hospital Clifton laboratory Karachi, Pakistan over a period of 7 months from 1st June until 31st Dec 2014. Healthy blood donors and patients requiring blood products of different age groups were employed. After an informed consent blood grouping was performed by taking blood samples under aseptic measures in EDTA and Gel separating serum tubes for ABO & Rh blood grouping by tube method.

Results: Total 1583 individuals included 868 (54.8%) males and 715 (45.2%) females. O+ve blood group found to be more common group (31.9%) followed by B+ve (31.2%). In Rh system, Rh+ve (97.2%) were more common than Rh-negative (2.8%) blood groups.

Conclusion: This study was taken out to maintain the blood product inventory concerning the management of blood bank and transfusion services for the patient.

FREQUENCY OF ABO BLOOD GROUP DISCREPANCIES IN TERTIARY CARE HOSPITAL KARACHI AND THEIR CLASSIFICATION INTO GROUPS

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Introduction: In developing countries, the blood grouping is generally being performed by the forward grouping method only. The aim of this study was to find out the frequency of ABO blood group discrepancies in tertiary care hospital in metropolitan city of Pakistan and also to classify these discrepancies on the etiological basis.

Methods: Cross-sectional, descriptive type of study was conducted at Blood Bank of Dr. Ziauddin Hospital Karachi, Pakistan. Total 1522 samples were included with exclusion of newborns and infants up to the age of 6 months. Blood samples were collected from a peripheral vein by trained phlebotomist and submitted to the blood bank department in 2 tubes, purple top vacutainer tube containing Di-potassium Ethylene Diamine Tetra Acetic Acid (K2 EDTA) anticoagulant and red top vacutainer tube containing clotting blood. Blood group testing was performed by tube technique.

Results: In total 1557 patients (854 Males and 703 Females) from various clinical specialties were included for ABO blood grouping. The male to female ratio was 1.2:1 (854 males and 703 females) with age ranging from 9 months to 92 Years. ABO discrepancies were Positive in 18 out of 1557 Patients (1.1%). Male to female ratio among ABO discrepancies were 1.5:1. There were four groups of ABO discrepancies. The most common group of discrepancies were group I discrepancies consisting of 12 out of 18 Patients (66.7%). Group II discrepancies were consist of 2 out of 18 Patients (11.1%). Group III discrepancies were consisting of 1 out of 18 Patients (5.5%) and Group IV discrepancies were consisting of 3 out of 18 Patients (16.7%).

Conclusion: The forward and reverse grouping are the essential parts of blood group testing for assigning the correct ABO blood group to the individual. ABO discrepancies i.e. forward and reverse grouping are not matches to each other can cause severe transfusion reactions which can be fatal for life. Furthermore, to ensure that correct ABO blood product to be transfused for saving life. Therefore reverse grouping should be strictly adopted in every

blood bank and transfusion services as a routine practice.

SELF MEDICATION PRACTICE AMONG UNDERGRADUATE MEDICAL STUDENTS

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Introduction and Objective: Assessment of self-medication practice among medical students is presumed to be of exceptional importance as in future they have promising role in counselling the patients regarding the rational use of drugs. This study was specially designed to evaluate self-medication practice in 3rd year medical students.

Methods: A cross sectional institution based study was conducted from Jan – March 2013 on 3rd year medical students at Bahria University Medical and Dental College, Karachi, after approval by ERB-BUMDC. Data was collected by questionnaire, filled on one to one basis after taking verbal consent and analysed on SPSS version 16.

Results: Frequency of self-medication was found to be 90%. Quick relief (38%) and busy schedule (21%) were the main reasons provoking self-medication. Common ailments that led to self-medication were: headache (95%) and fever (87%). Drug groups frequently used were: analgesics (95%), antipyretics (87%) and antibiotics (36%). Acetaminophen was the most common analgesic (73%) as well as the antipyretic (88%) while antibiotics were, metro-nidazole (26%) followed by amoxicillin plus clavulanic acid (17.3%).

Conclusion: Practice of self-medication is high among medical students. Potential problems of self-medication should be emphasized to the students.

DETERMINATION OF PROSTATE GLAND VOLUME BY ULTRASONOGRAPHY AND ITS CORRELATION WITH ANTHROPOMETRIC MEASUREMENTS IN A SUBSET OF KARACHI POPULATION

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Introduction and Objectives: To establish the local reference range of prostate volume according to our subset of population. To correlate prostate volume (PV) with age, body mass index (BMI) and waist circumference (WC).

Methods: A cross-sectional study with 119 healthy adults aged 40-79 years without any prostatic pathology were recruited. The study population was categorized into 4 age groups (40 - < 50 yrs, 50 - < 60 yrs, 60 - < 70 yrs, 70 - < 80 yrs), 3 BMI groups (healthy, obese and overweight) and 2 WC groups (< 90cm and > 90cm). A p-value of < 0.05 was considered significant. A total of 130 healthy adult volunteers were recruited initially, out of these 20 individuals were those whose IPSS was < 8 and prostate volume on ultrasound was > 25 ml. These 20 subjects underwent Uroflowmetry (UFM). After UFM, total 19 subjects were labelled as healthy adults and 1 subject was excluded from healthy adult group. The sample of 119 healthy subjects were studied sonographically for the effects of Age, BMI and WC on prostate volume.

Results: The mean prostate volume was 21.7±2.2mls, mean body mass index was 28±6kg/m², whereas mean waist circumference was 95cm. PV was found to be higher in obese and > 90cm waist circumference group. After applying multiple regression analysis, waist circumference correlated positively and significantly with prostate volume. UFM showed negative correlation with IPSS and PV.

Conclusions: Mean prostate volume in our studied population was smaller than that of many western populations. Our study has proved that central obesity is the most important factor influencing prostate volume.

ASSOCIATION OF KISSPEPTIN, ENDOMETRIAL RECEPTIVITY AND UNEXPLAINED INFERTILITY

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Introduction and Objectives: To relate serum Kisspeptin levels with pregnancy outcome after intra cytoplasmic sperm injection (ICSI).

Methods: In the cross sectional survey carried at Australian Concept Infertility Medical Centre from June 2014 to June 2015, 176 females with 20 to 42 years of age and with regular menstrual cycles were included for ICSI. Patients with uterine fibroids and metabolic disorders were excluded. Down regulation of ovaries was followed by controlled ovarian stimulation, ovulation induction, oocyte retrieval, microinjection and embryo transfer. Serum samples for estimation of Kisspeptin, was done on OI day and endometrial thickness was measured. Based on beta hCG results were categorized into group A, non-pregnant with beta hCG < 25 mIU/ml II, and group B, clinical pregnancy with beta hCG > 5 mIU/ml.

Results: Kisspeptin levels were significantly higher in the Group B versus Group A ($p < 0.001$) independently associated with positive pregnancy ($r = 0.388$; $p < 0.001$), and endometrial thickness ($r = 0.294$; $p = 0.05$) irrespective of the age and BMI of the subjects.

Conclusion: Kisspeptin is a positive pregnancy marker in females after ICSI as a result of its effects on oocyte maturation and endometrial thickness.

EFFECT OF LONG HOUR SHIFTS ON MEDICAL INTERNS AND RESIDENTS, AND THE STRATEGIES ADOPTED TO COPE WITH WORK-RELATED STRESS

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Introduction and Objectives: To assess the impact of long hour shifts on performance and health of medical interns and residents and strategies adopted to cope with stress.

Method: This cross-sectional study was conducted from January to July 2015. A sample of 200 interns and residents was taken from Ziauddin Hospital Clifton, Kemari and North Nazimabad. Data was collected through a self-designed questionnaire and stress levels assessed through General Health questionnaire (GHQ-12). Data was analyzed through SPSS v 20. Chi-square and Paired T test was applied.

Results: The mean age of participants was 27+ 2.3 years. Average sleep hours when doing call were 3.06 +1.47. Work performance was below average in 62 % of the population. Stress was found in 34% of study population. Increased tea intake was the stress coping strategy adopted by 72 %. There was a significant association between stress level, gender and personal habits. ($p < 0.05$). A significant difference was found between cigarette and energy drink intake before and after starting job ($p < 0.05$).

Conclusion: High stress may have negative effects on health and performance. Hence, medical interns and residents need support to cope with stress.

RECURRENT MISCARRIAGE AND ASSOCIATED FACTORS

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The incidence of recurrent pregnancy loss in women of reproductive age group is 0.5%–3%, and idiopathic causes accounts for 50%–60% of recurrent pregnancy losses. Approximately 30%–50% of conceptions end in spontaneous miscarriage before the completion of first trimester. Miscarriages mostly occur at the time of implantation. The causes of recurrent pregnancy loss are many including parental chromosomal abnormalities, maternal thrombophilias, immunologic causes and endocrine disorders. Recurrent pregnancy loss is an extremely distressing clinical problem for women as well as health professionals. According to current studies

the decidualized endometrium act as a biosensor of good quality embryos, if it is faulty, may lead to implantation of embryos resulting in miscarriage. Other factors implicated in the pathophysiology of miscarriage are Systemic and placental oxidative stress. Vascular endothelial damage, abnormal vascularity of placenta and immunologic reactions have been proposed to play some role in causing recurrent miscarriage.

The objective of this review is to discuss the causes of recurrent miscarriage, based on the published research articles.

PREVALENCE AND PREVENTIVE MEASURES OF PROSTATE CANCER: A RANDOMIZED STUDY IN PUBLIC AND PRIVATE HEALTH CARE SECTOR

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Introduction and Objectives: Prostate cancer accounts for abnormal or uncontrolled division of cells of prostate in male. It is a slow growing cancer which is usually localized rarely metastasized. The growth rate varies from slow moderate to high. It mostly occurs in old age and depends on many other factors like weight, height, diet, habit, environment, genetics, and sexual disease. This study aims to evaluate occurrence, causes, problems associated with disease and treatment of this cancer in view of the fact to evaluate consequences responsible to contribute carcinoma of prostate.

Methods: For this purpose randomized study conducted at public and private health care sector (n= 100 patients of prostate cancer).

Results: The Gleason score are used to diagnose prostate cancer, 10% of patients have score 5 (4+1), 40% of patients have score 7 (4+3) or (3+4), 44% of patients have score 9 (5+4), 6% of patients have score 11 (5+6) which shows that mostly prognostic grade II, III IV, V occur. Mostly traditional prostatectomy is used as a treatment and 78% of the patients got benefit from it.

Conclusions: This study concludes that the prostate cancer may occur due to age factor (old age), fatty diet, tobacco or alcohol intake. Finding of cancer at score 5,7,9,11 and associated complications are hypertension, urinary tract infection, kidney stones.

KNOWLEDGE AND ATTITUDE TOWARDS MENOPAUSE IN WOMEN AGED 45-60 YEARS

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Introduction and Objectives: Menopause has significant physical, mental, social and psychological effects on women's life and prior knowledge about these changes help them to cope with these changes with greater readiness. Educating women regarding menopausal changes would definitely equip them better to tolerate these menopausal symptoms.

Methods: A cross sectional study based on sample of convenience was conducted at the outpatient department of Ziauddin Hospital, Karachi from 1st July 2014 to 31st January 2015. The study participants were 250 menopausal women between the ages of 45-60 years. Data was collected by means of a questionnaire, which consists of three parts containing questions about demographic information, knowledge and attitude.

Results: The mean age of respondents in the current study was 51.9 +/- 4.2years. The mean age at menopause was 45.9 +/-3.8years. As far as the knowledge about menopause is concerned 4.8% had weak, 71.2% had moderate and 24% had good knowledge about menopause phenomenon. Majority of respondents had positive (72.4%) attitude towards menopause and 27.6% had negative attitude towards menopause.

Conclusions: Appropriate knowledge and positive perception towards menopause is very important to help women to cope with menopause transition and can help in the development of

appropriate programmes to promote women health.

SOCIAL SUPPORT AS A DETERMINANT OF INTERNALIZED STIGMA AND SELF-ESTEEM IN PERSONS WITH SCHIZOPHRENIA

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Introduction and Objectives: Social support works as a buffering factor in psychotherapeutic treatment for the diagnosed persons with schizophrenia in reducing internalized stigma and in boosting their self-esteem. The aim of the present study is to explore the predictive relationship of social support with internalized stigma and self-esteem among diagnosed persons with schizophrenia.

Methods: A purposive sample of 52 diagnosed persons with schizophrenia, age ranges 18 to 55 years who belong to different socioeconomic status was taken from different psychiatric hospitals of Karachi. Demographic Information sheet, Multi-Dimensional Scale of Perceived Social Support, Internalized Stigma of Mental Illness Scale (ISMI) and Rosenberg Self Esteem Scale were administered individually.

Results: Findings revealed social support predicts internalized stigma and self-esteem in persons with schizophrenia ($p < 0.000$).

Conclusions: There is an immense need to develop awareness regarding social support group and to generate strategies to reduce the effect of internalized stigma in person with severe mental illness.

EFFICACY AND SAFETY OF METFORMIN IN GESTATIONAL DIABETES MELLITUS

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Objective: To assess the efficacy and safety of Metformin for the management of gestational diabetes Mellitus (24 wks and above) as compared to Diet.

Methods: This Quasi experimental study is being conducted on 600 females at Ziauddin University Hospital. All pregnant women receiving prenatal care fulfilling the inclusive criteria will be screened using a 50g glucose challenge test (GCT). After the result if it is positive, women will be advised for oral glucose tolerance test. After diagnosis of gestational diabetes Mellitus patients will be advised for diet control or oral hypoglycemic drug metformin starting from 500mg twice a day. Patient will be followed fortnightly with sugar record for adjustment of treatment. Patient will be followed till delivery for any maternal or perinatal complications. Patients with systemic diseases are excluded from the study.

Results: Result of this study are awaited as this is an ongoing study.

Conclusions: The main purpose of the treatment is to prevent fetal hyperinsulinemia and fetal macrosomia by reducing maternal glucose level, decrease risk of appearance of diabetes in subsequent pregnancies and in future without pregnancy. Metformin is an alternative to insulin and is effective in the treatment of gestational diabetes.

IMPACT OF PHARMACIST INTERVENTION ON ANTIBIOTIC PRESCRIPTION ORDERS IN PEDIATRIC SETTING

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Introduction and Objective: The prospective study was designed to formulate and test the efficacy and impact of trial interventions by pharmacist on potential harm and incidence rate of errant antibiotic medications dispensed in children admitted to a public sector hospital in Karachi.

Methods: An eight months study was conducted in a teaching hospital comprising of 850 beds with 65 beds strength in three pediatric units I, II and III inclusive of neonatal ICU. Orders written by physician groups in all patient admission categories were

included. The prescription order review prior to dispensing was made by experienced and specialized pharmacists in pediatric pharmacotherapy in a research setting. Prescription order in error was marked if not in compliance with standard pediatric references, dosing guidelines and antibiotic prescription guide adopted in the hospital. As per predefined criterion in our study, errors were labeled as potentially lethal, serious and significant. The data was statistically compared by unpaired samples test and the efficacy of the intervention on type of errors was margined by chi square test.

Results: 8.5 medication errors per 1000 prescription orders were identified (1.5 medication error per 50 patient per day), where-as 18 potentially lethal errors were identified within the span of 8 months. Potential cardiopulmonary arrest and anaphylactic risk was significantly assessed ($p < 0.01$) in ICU. Overdose and under dose of antibiotics accounted to 76% errors. The frequency of errors was comparatively non significant in prescription orders by senior attendees ($p < 0.05$).

Conclusions: Effective control of antibiotic medication errors is observed with the integrated role of pharmacist in the prescription-dispensing-administration sequence.

SIMULTANEOUS DETERMINATION OF SIX ANTIHISTAMINE ANTIALLERGIC DRUGS IN PHARMACEUTICAL FORMULATIONS, HUMAN SERUM AND PHARMACOKINETICS APPLICATION

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This article describes a new, accurate and highly specific high performance liquid chromatographic method with UV detection (HPLC-UV) for the simultaneous determination of cetirizine HCl, chlorphenamine maleate, loratadine, domperidone, buclizine and meclizine in pharmaceutical dosage form and human serum, involving pyridoxine as internal standard. The mobile phase consists of heptane sulphonic acid salt buffer and acetonitrile, drawn at a flow rate of 1.0 mL/min using a symmetry C18 column with UV detection at 230 nm. The intraday and inter-day precision measurements showed coefficients of variation always less than one. The calibration curve was tested in the range of 10–2150 ng/mL and the correlation coefficient of > 0.9990 in all cases was obtained. The averages of the absolute and relative recoveries were found to be in the range of 98 to 102%. Up to six drugs were separated in the same chromatogram with good resolution. The proposed HPLC method has reasonable applications in pharmaceutical tablet dosage form and pharmacokinetics studies.

SIMULATION BASED CARDIOPULMONARY AUSCULTATION: A TOOL TO AID MEDICAL STUDENTS OF ZIAUDDIN UNIVERSITY

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Introduction and Objectives: Use of simulation and state of art mannequin and models in clinical skills lab is now becoming a common practice which is offered to 3rd year medical students during their clinical rotations. We seek to determine the efficacy of cardiopulmonary auscultatory skills in 3rd year medical students of Ziauddin University who have been trained by simulation based auscultatory device.

Methods: Students who participated in this study ($n=40$) were from 3rd year MBBS program. They were assigned in 2 equal groups. Group A took a lecture on the detection and knowledge of various cardiopulmonary sounds whereas Group B was tutored on a simulation based device after they had taken their lecture. After respective training sessions, each group was tested with the help of a questionnaire. This questionnaire consisted of identification and knowledge based open ended and close ended questions. Data

was entered into data analysis software S.P.S.S ver 15, Data was analyzed and mean, standard deviation was calculated along with Charts, graphs were generated to represent results.

Results: Diagnostic accuracy and correct identification of cardiopulmonary sounds was better in the group that had been assisted with a simulation based device. Mean score of assessment of knowledge and skills of cardiopulmonary sounds were found significant in group B (mean = 13.5 ± 1.73) compared to group A (mean = 6.9 ± 2.64).

Conclusions: According to our study at Ziauddin medical university, it is suggested that simulation based learning is a subsidiary tool in preserving knowledge and skills for cardiopulmonary auscultation.

WHOLE FRUIT BETTER THAN FRUIT JUICES IN MAINTAINING BLOOD SUGAR LEVELS

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Introduction and Objectives: Type 2 diabetes mellitus (T2DM), non-insulin dependent diabetes mellitus, is the most rapidly growing disease, estimated to affect 439million adults by 2030. The rate is faster in developing countries than developed countries which in turn results in higher healthcare expenditure. Thus it becomes a public health priority to prevent the disease and control over its rapid growth. Among the known risk factors that contribute to T2DM are dietary factors, which promote early diabetes in healthy individuals and increased mortality and morbidity in type 2 diabetic patients. To evaluate the hypothesis that fruits provide more protection against T2DM than fruit juice, we performed experiment by giving fruits to one group and fruit juice to another group of healthy volunteers and monitor fasting blood sugar and random blood sugar.

Results: The results of current study suggested that whole grapefruit and orange fruit are very effective in maintaining random blood sugar levels in comparison to juices.

Results also suggested that dietary fibers are not only effective in regulating blood sugar, but also showed insignificant change in systolic and diastolic blood pressure and also in pulse rate. Moreover, whole grapefruit has been found more effective in maintaining pulse rate in comparison to fibers obtained from apple fruit and orange fruit.

Conclusions: Based on above findings, it has been concluded that patients with type 2 DM could be maintained on dietary fibers not only for better glycemic control, but also for controlling diabetes related complication such as high blood pressure. This study could further be extended to assess the effects of dietary fibers (especially obtained from grapefruit) in controlling other diabetes related complications, e.g. hypercholesterolemia, diabetic nephropathy, in diabetic patients.

NAFLD IN DIABETIC PATIENTS TREATED ON ORAL HYPOGLYCEMIC.

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Introduction and Objectives: Determination of NAFLD (non-alcoholic fatty liver disease) in diabetic patients on oral hypoglycemic therapy. Diabetes is one of the most common non-communicable disorder throughout the world. It has enormous complications, which indirectly multiply the economic and medical burden. According to WHO, 9% population of the world suffers from Diabetes who are above 18 years age. Prevalence of diabetes in Pakistan is about 10% according to Akhtar. This poses a huge burden on a country like Pakistan, which already has very limited resources in field of health.

Deficiency of Insulin causes hyperglycemia and hyperlipidemia. This leads to multiple disorders like atherosclerosis, CVAs, IHD, Renal failure, NAFLD, glaucoma, cataracts, gangrenes of limbs, acute emergencies like DKA (diabetic ketoacidosis) and HONKC (hyperosmolar non ketotic coma) etc. NAFLD (Non Alcoholic Fatty Liver Disease) was first described by Ludwig in 1980 and is on rise, making it the most common cause of CLD. Commonly called fatty liver diseases is a major problem arising in diabetic population, which has been neglected off until now. The prevalence rises to 60.8% in diabetics in Pakistan. 40% cases of NAFLD can progress to NASH (nonalcoholic steatohepatitis) of whom 32%-37% can progress to advanced fibrosis. This prevalence rises to 35% to 75% in obese and diabetics. Making it most common cause of cryptogenic cirrhosis, which can lead to hepatocellular carcinoma. NAFLD is usually an incidental finding when an abdominal scan is done for some other suspected pathology.

Methods: FBS, Fasting LIPID profile and HbA1C was done. Ultrasound was done to see fatty infiltration of liver.

Conclusion: Ultrasound liver of type II diabetic patients showing non-alcohol fatty liver changes.

IDENTIFYING ERRORS IN PRESCRIPTION WRITING

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Introduction and Objective: The errors of prescribing are the commonest form of avoidable medication errors and are considered to be the most important target for improvement. Medication errors are common in general practice. Both errors in the act of writing (prescription errors) and prescribing faults due to erroneous medical decisions can result in harm to patients. Our objective is to identify the errors in the prescriptions of general practitioners (GPx) from different parts of Karachi.

Methods: A descriptive pilot study was conducted from 1st January to 30th February 2014. A total of 100 prescriptions were collected from 4 general practitioners' clinics (east, west, central and south districts) of Karachi. Verbal consent was taken few days prior to random collection. All prescriptions were analysed for errors in superscription, inscription, subscription, transcription, signatures and refill information.

Results: A total of 373 errors were identified in 100 prescriptions. 203 errors in superscription, 6 in subscription, 34 in transcription, 1 in prescribers' signatures and 62 in refill information.

Conclusion: Errors in prescription writing are found to be common in the prescriptions of general practitioners. Measures should be taken to refresh the prescription writing skills of general practitioners through CMEs and workshops.

ASSESSMENT OF STRESSORS AMONG UNDERGRADUATE DENTAL STUDENTS OF KARACHI, PAKISTAN

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Introduction and Objectives: Dental schools are known to have highly stressful learning environments. Dentistry involves an acquisition of required academic, clinical and interpersonal skills during the course of learning. Identifying sources of stress represents the crucial first step towards advocating policy changes and enhance student's stress coping skills. This study was conducted to assess the problems related to stress encountered by dental students of Karachi. The aims are: a) To identify the common stressors among undergraduate dental students of Karachi. b) To investigate whether specific stressor is related to year of study or gender. c) To evaluate the factors which influence student perception that might allow programmatic changes.

Methods: Type of Study: Cross Sectional Survey; Sample Size: 330 undergraduate dental students of Karachi; Study population: second, third and final year BDS; Study setting: Five dental schools of Karachi; Modified form of Dental Environment Stress

questionnaire was administered.

Results: A total of 500 Questionnaire were distributed out of which only 330 responded. Of the respondent, 70% were women and 30% were men. The most stressful variable among all was 'Overloaded feeling due to vast syllabus'. Third and final year students were reported to have higher-level stress than second year students. Females were more affected by the same stressor than male students.

Conclusions: The conclusion of study was drawn from variables with significant values <0.005 . Stress due to accommodation problem was higher in students of second year while clinical based problem caused greater level of stress among students of third and final year. The variables relating to academics (inadequate break timings, inadequate time for assigned work and overloaded due to vast syllabus) caused stress in all three years equally. Stress showed an upward trend from the first to the final year with a jump from the second to the third years.

FREQUENCY OF DEPRESSION IN HOUSE WIVES AND ASSOCIATED RISK FACTORS

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Introduction and Objectives: In any population, women are more prone to depression. In our middle class community it is still considered a taboo. Housewives usually emphasize on somatic issues rather than emotional signs and symptoms which causes detrimental effects on their health. Timely identification of risk factors and counseling of women regarding their mental health is of utmost importance. We aim to measure frequency and identify major risk factors of depression in housewives belonging to middle class community in Karachi.

Methods: Cross sectional study conducted on housewives between 25-50 years of age. Through convenience sampling technique 247 questionnaires with 17 questions were administered, followed by the patient's health questionnaire 9 (PHQ-9). Data was analyzed by SPSS v20. Chi-test was calculated for categorical data and p-value <0.05 was considered significant.

Results: Out of all the respondents, 131 females (53%) turned out to be depressed and 116 (57%) not depressed. PHQ-9 classification of the levels of depression in the participants showed 100 females mildly depressed, 77 females moderately depressed, 35 females moderately-severely depressed, 16 females severely depressed and 19 females not depressed.

Conclusions: Most prominent risk factors for depression were of having to take care of an old or an ill person at home and living in a joint family structure. Although the study targeted middle class community, the analysis showed no associations with the family income.

FREQUENCY AND TYPE OF CHEWABLE TOBACCO COMMONLY USED BY ORAL SQUAMOUS CELL CARCINOMA PATIENTS

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Introduction: South East Asia is threatened by the risk of Oral Squamous Cell Carcinoma. The popularity of chewable tobacco plays a pivotal role in making it the second leading malignancy in Pakistan. Prolonged use of these products can lead to different oral precancerous conditions, GI cancer, ulceration and many other diseases. The objective of the study was to find out the frequency and type of chewable tobacco, which has been the cause of Oral Squamous Cell Carcinoma in these patients.

Methods: The study was descriptive. Seventy one patients who had Oral Squamous Cell Carcinoma and a history of tobacco use were selected from cancer OPD. Subjects addicted to stuff other than smoking and chewable tobacco were excluded. A questionnaire was used to collect demographic characteristics, oral hygiene, medical history, family history of cancer, and detailed

history of tobacco usage, chewable or smoke products and details of chewing habits (using since, packs/day, duration of exposure etc.). The study was carried out in Ziauddin.

Results: Majority of the subjects were males (80%). Out of the 71 Oral Squamous Cell Carcinoma patients majority 42 (59.15%) were multiple users that consumed more than one tobacco product. Whereas, 9 (12.67%) consumed gutka, 9 (12.67%) pan, 5 (7%) betel nut, 4 (5.6%) niswar and 2 (2.8%) cigarette.

Conclusions: Frequency of OSCC was found higher in users of multiple chewable tobacco formulations which makes consumption of multiple tobacco formulations one of the major etiological factors in the development of OSCC.

MOLECULAR JOURNEY OF VITAMIN D, VDR AND VDRGP AND ITS ASSOCIATION WITH BREAST CANCER

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Vitamin D plays an essential role in regulating the levels of calcium and phosphate in the body. Vitamin D is not active as a hormone - instead, a few chemical changes must be made. These are performed by enzymes that add hydroxyl groups. Vitamin D receptor is a nuclear hormone receptor Transcriptional regulator in response to 1,25-dihydroxyvitamin D 3.

VDR protein is at the centre of the Vitamin D endocrine system with a negative feedback which regulates serum calcium and Vitamin D, if not maintained will affect large number of organs including breast. Data is available in positive genetic association of certain diseases which suggest functional consequences of VDR gene polymorphism. Vitamin D plays a role in breast cancer and exerts its effect through VDR gene polymorphism. There are four polymorphic variants of VDR (Folk 1, Bsm1, Taq 1 and Appa1). Hormone Receptor-Vitamin D hormone binds to receptors in its target cells, controlling the synthesis of many different proteins involved in calcium transport and utilization. The receptor is composed of two domains: a domain that binds to the hormone and a domain that binds to DNA. It pairs up with a similar protein, 9-cis retinoic acid receptor (RXR), and together they bind to the DNA, activating synthesis in some cases and repressing it in others. There is a decrease in VDR expression in breast cancer cells compared to normal breast cells and this decrease could be due to gene polymorphism and other factors also Alterations in VDR expression and activity could lead degranulation of vitamin D uptake, metabolism and serum levels of biologically active Vitamin D.

POTENTIAL OF BLACK MULBERRY: FROM FOOD OF CHOICE TO REMEDY FOR BOWEL DYSMOTILITY

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Introduction and Objectives: *Morus nigra* Linn. (black mulberry) is used in gastrointestinal ailments. This study demonstrates gut modulatory properties of *M. nigra*.

Methods: The prokinetic, laxative and antidiarrheal activities of *M. nigra* were assessed in mice, while isolated rabbit jejunum and guinea-pig ileum were used to explore insight into mechanism(s).

Results: At 30 and 70 mg/kg, the crude extract of *M. nigra* (Mn. Cr) exhibited atropine-sensitive prokinetic and laxative effects, similar to carbachol (CCh). While at higher doses (100-500 mg/kg), Mn.Cr offered protection against castor oil-induced diarrhea.

In rabbit jejunum, Mn.Cr and its chloroform fraction inhibited CCh-induced contractions more potently compared to high K⁺ (80 mM). Conversely, petroleum fraction was more potent against high K⁺-induced contractions. At 0.01 mg/mL, Mn.Cr caused a parallel shift in acetylcholine (ACh) concentration-response curves (CRCs) followed by a non-parallel shift at 0.03 mg/mL, similar to

dicyclomine. At further tested concentrations, Mn.Cr (0.1 and 0.3 mg/mL) and petroleum fraction suppressed Ca²⁺ CRCs, similar to verapamil. In guinea-pig ileum, Mn.Cr, its aqueous and ethyl acetate fractions exhibited atropine-sensitive gut stimulant activity along with additional uncharacterized excitatory response in aqueous fraction only.

Conclusions: These results suggest that black mulberry possesses prokinetic, laxative and antidiarrheal effects, putatively mediated through cholinomimetic and anti-muscarinic plus Ca²⁺ +-antagonist mechanisms, respectively.

ANTIBACTERIAL ACTIVITY OF HONEY AND TURMERIC AGAINST GIT AND RESPIRATORY

PATHOGENS

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Introduction and Objectives: Because of emergence of resistant pathogens the antibiotic activity is diminished. This poses a serious threat to public health. Therefore alternative antimicrobial techniques are needed. Thus this situation has led to reevaluation of the therapeutic use of ancient remedies such as plants and plant based products including honey and turmeric.

Methods: The study was performed to evaluate the antimicrobial activity of honey and turmeric, purchased from local market. Different concentrations of honey and turmeric were prepared in distilled water including 100%, 75% and 50%. Its effect was observed on isolates obtained from clinical samples, which includes *Salmonella typhi*, *Shigella dysentrey*, *S.aureus*, *Proteus mirabilis*, *E.coli*, *Pseudomonas aerignosa*, *Klebsiella oxytoca*, *Bacillus subtilis*, *Enterococcus* and *Micrococcus leutus*. The study was carried by disc diffusion method. All experiments were performed in triplicate. Zone of inhibition was measured in millimeter and results were given in percentage.

Results: Honey extract show highest antibacterial activity as compared to turmeric extract. Highest concentration (100%) of honey and turmeric was inhibitory to all strains among which *Enterococcus* gave 75% inhibition while other strains were inhibited up to 50%. It was found that 75% concentration of these compounds gave intermediate zone of inhibition while 50% concentration was least inhibitory.

Conclusions: Honey and turmeric have potential to use as therapeutic agents either alone or in combination against various infections.