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Plenary

Next Gen Sequencing Revolutionizes Molecular Diagnostics and Translational Medicine

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When Watson and Crick published their seminal paper on the structure of DNA in Nature in 1953, they inserted the line, "It has not escaped our notice that the specific pairing that we have postulated immediately suggests a possible copying mechanism for the genetic material." This was the forerunner of a revolution, which through PCR and Sanger sequencing changed the face of molecular diagnosis of Mendelian disorders and infectious diseases. Genetic heterogeneity led to sequential sequencing of many genes to arrive at a specific diagnosis, and was therefore expensive. Sanger sequencing of large genes like dystrophin and NF1 was also expensive. However, the advent of next gen sequencing brought down the cost of analysis and by allowing us to study many genes or the whole genome together at one go ushered the modern revolution in genomics and its many applications for improving human health.

My presentation will describe how the new bio-technologies, including next gen sequencing, have started to be applied to patient care in India. This has brought these high-end technologies to the bedside and the clinical table. Practical examples will illustrate the application of these new technologies to genetic disorders and microbiology. I will emphasize genotype driven personalized therapies in cancer and other genetic disorders, genetic testing in those at risk as revealed by family history, pharmaco-genomic testing in clinical practice, use of panel testing of multiple genes, exome sequencing and whole genomic sequencing to diagnose "unknown disorders", and finally using NGS to advise about risk for complex disorders like coronary artery diseases and diabetes mellitus. I will also point out the limitations of these technologies as applied in India, and how their judicious use can change the practice of medicine.

Albumin Matters

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Albumin has been in the scientific literature for over 2000 years since Hippocrates observed foamy urine in renal disease patients. More recent milestones include the first recorded dialysis in 1840 and the Starling's elucidation in 1896 of albumin's role in maintaining circulation and the discovery of its gene sequence in 1986. Today we have a thorough understanding of albumin's structure, synthesis and homeostasis and we can measure it precisely but not necessarily accurately since standardization is lacking. That albumin measurement has an important place in assessment of chronic renal and liver disease and nutritional status is not disputed although it's place as a marker of all-cause mortality is less well recognized. Also under recognized is that commonly used routine methods for albumin measurement can produce wildly different results depending on the clinical context. Alarmingly, differences are greatest in those who are most unwell; bromocresol green (BCG) based albumin methods significantly over-estimate albumin concentration in such individuals. Using a less specific method such as BCG could lead to inappropriate patient management and poor outcomes. Laboratories have a responsibility to provide assays that are fit for purpose and to understand their assay's limitations.

Awadesh Saran Memorial Oration

Pharmacogenomics: Implementation in Medicine

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Recent years have seen significant increase in the pharmaceutical industries globally, which has also witnessed an increase in the incidence of adverse drug reactions. With the inherent inter-individual genetic variations leading to altered drug response, the field of pharmacogenomics or personalized medicine, or precision medicine has gained immense importance in medicine in all stages of healthcare including prevention, diagnosis, treatment and follow-up. Various genetic tools are employed to identify genetic variants responsible for altered drug response in individuals in order to improve drug safety, optimize efficacy and to reduce adverse drug reactions.

In India, studies have been aimed in establishing the frequency of variant allele of polymorphic genes involved in drug metabolizing enzymes especially from the Cytochrome P450 s superfamily. Further, the knowledge of pharmacogenomics has been implemented in different areas of medicine primarily in field of oncology. However, one of the challenges that face the field of pharmacogenomics in India is its population, which is ethnically, culturally and genetically quite different and so is the variant allele frequencies. Also the altered drug response is primarily due to the genetic variation; however other environmental factors can also contribute

to the drug response variability, which needs to be taken in consideration.

Nevertheless, the increased understanding due to high throughput technologies such as the next generation sequencing (NGS) of the genetic susceptibility along with the greater support from the government agencies like DBT, DST and ICMR together holds great promises in the implementation of pharmacogenomics in medicine in the near future.

Dr. Taranath Shetty Memorial Oration Popular Lecture

Emerging Concepts in Pathophysiology of T2DM

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Diabetes is the most common non-communicable disease globally and has assumed epidemic proportions both in developed and developing countries. The recent data IDF of global estimates shows that 387 million people were afflicted with diabetes by 2014, and this number is going to be ballooned to 592 million by 2035. Great number of the people having diabetes is between 40 and 59 years of age. Type 2 diabetes is characterized by two defects: insulin resistance and insulin deficiency. The growing prevalence of obesity, adaptation to sedentary life style, consumption of calorie-dense food and increasing longevity (increased adipose tissue mass) and possibly genetic factors have contributed to increasing insulin resistance. β cells of the pancreas have a tremendous capacity to overcome this increasing insulin resistance, but eventually they exhaust and manifest as hyperglycemia due to decline in insulin secretion. However, after the onset of hyperglycemia, the decline in β cell function is rapid, progressive and inexorable. The ongoing glucotoxicity, lipotoxicity, oxidative stress and enhanced programmed β cell apoptosis leads to β cell dysfunction. Last few years have witnessed and important role of incretins in the pathophysiology of T2DM. Progressive decline in incretins particularly in GLP1 and GIP results in decrease in insulin secretion and worsening of hyperglycemia. The uses of DPPIV inhibitor and GLP analogues have modulated the whole scenario. In addition, newer understandings in programmed β cell death has also resulted in exploration of new therapeutic targets.

K. L. Gupta Memorial Oration

Circumventing Cancer Drug Resistance in the Era of Personalized Medicine

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Resistance to systemic therapy (refractory tumors) and metastasis (recurrent tumors) pose major clinical challenge in successful treatment of cancer and account for more than 90% of cancer-associated deaths. Although, tumor genome sequencing has provided powerful tool for cataloging cancer driver-mutating genes, it cannot distinguish those genes that drive tumor progression or metastasis. In addition to genetic alterations early-stage tumors require some ancillary changes to become invasive and to metastasize. Many inflammatory mediators produced in the tumor milieu can support tumor growth and cell survival to promote metastatic competence.

Using genomic and proteomic approaches, we identified *TGM2* as one of the highly overexpressed genes in multiple drug-resistant and metastatic tumors. *TGM2* is a stress response gene, which encodes a functionally and structurally complex protein, called transglutaminase type II (TG2). Stable expression of TG2 bestows cancer cells with increased ability to invade and survive the toxic effects of radiation/chemotherapy by activating inflammatory signaling networks. TG2 over expression induced epithelial-to-mesenchymal transition (EMT) and conferred stem cell traits in epithelial cancer cells. At molecular level, TG2 expression resulted in constitutive activation of NF-κB via a non-canonical pathway. Hypoxia-induced factor-alpha (HIF-1α) was identified as one of the downstream targets of TG2-induced NF-κB activation. The resultant increase in HIF-1 accumulation led to transcriptional regulation of Snail, Zeb, and Twist repressors. Importantly, elevated expression of TG2 in tumor samples is associated with poor disease outcome, increased incidence of metastasis, and early relapses. Taken together, these results suggest that inhibition of TG2 represents a promising approach to intervene cancer progression and to reverse chemoresistance. As a proof-of-concept, our studies provided compelling evidence that in vivo silencing of TG2 by liposomal-TG2siRNA (LSRNA) could effectively inhibit the dissemination of orthotopically growing tumors (pancreatic and ovarian) and render them sensitive to chemotherapeutic drugs. In view of the usefulness and efficacy of LSRNA to inhibit TG2 in growing tumors, efforts are underway to develop it for treating patients with refractory and recurrent tumors.

Mrs. & Dr. G. P. Talwar Oration

Vitamone D: Rationalization vs Sensationalization

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Vitamin D or cholicalciferol is a unique vitamin in the sense that apart from the dietary sources, it can be synthesized by our body through direct sun exposure. It is also recognized as pro-hormone or vitamone because it is synthesized in skin and transported to other sites for its action via vitamin D receptors present on various tissues. In the past decade, interest in this particular vitamin grown up due to identification of multi-systemic role played by it apart from calcium homeostasis. It led to increased interest in knowing the serum vitamin D levels globally in healthy individuals as well as with various systemic disorders. Through vast number of studies it has been proven that Vitamin D deficiency is associated with multiple systemic disorders viz. Cardiovascular Diseases, Metabolic syndrome, Diabetes, various Cancers, Autoimmune disorders, Materno-fetal outcomes etc. It has also been observed that vitamin D deficiency is quite prevalent in healthy population as well. In country like India, according to reference range of western countries more than 50% of the healthy population too is vitamin D deficient. There are numerous causes of vitamin D deficiency like decreased outdoor activity, environmental pollution, overclothing, injudicious use of sunscreen lotions with higher SPF, darker skin, old age, decreased dietary intake, genetic variations in various vitamin carrier protein and

metabolizing enzymes etc. Even though popularly known that 90% of Vitamin D comes from sunlight exposure, vitamin D supplement have been widely advertised and practiced, in part to avoid complications like sun-burns, skin cancers and possible defect in vitamin D metabolism pathway. Supplementations with vitamin D have resulted in improved serum vitamin D levels. They have been widely used as supplements for prevention and aid in treatment of many disorders. Data related to their effect on disease outcome have started to come in past few years. Meta-analysis of various randomized controlled trials reveals that even though vitamin D supplementation result in improvement in vitamin D status, but there result in improvising the overall morbidity and mortality is not convincing. Results of Clinical trials are in there early stage, multiple shortcomings of these early trials needs to be identified. It will be keen to watch how fair vitamin D play its role in becoming a miracle drug.

Dr. T. N. Pattabiraman Oration

Transcription Factor C/EBP-β Mediates Down-Regulation of Dipeptidyl-Peptidase III Expression by Interleukin 6 in Human Glioblastoma Cells

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Dipeptidyl-peptidase III (DPP III), is a cytosolic metallo-aminopeptidase implicated in various physiological and pathological processes. A previous study from our laboratory indicated elevated expression of DPP III in glioblastoma (U87MG) cells. In the present study we investigated the role of IL-6, a pleiotropic cytokine produced by glial tumors in regulation of DPP III expression. Immunohistochemistry, western blotting and qRT-PCR were used for quantitation of DPP III and IL-6 in human glioblastoma cells and tumors. Cell transfections and DPP III promoter reporter assays were performed to study the transcriptional regulation of DPP III by IL-6. Promoter deletion analysis, site directed mutagenesis, Chromatin Immunoprecipitation (ChIP) assays and siRNA technology was employed to elucidate the molecular mechanism of IL-6 mediated regulation of DPP III in glioblastoma cells. Our results for the first time demonstrate a negative correlation($r = 0.632$, $p = 0.01$) between DPP III and IL-6 in both human tumors and cultured glioblastoma cells. Treatment of U87MG cells with IL-6 significantly decreased DPP III expression with a concomitant increase in the levels of transcription factor C/EBP-β. Deletion/mutagenesis of C/EBP-β binding motif of DPP III promoter significantly increased its activity and abolished its responsiveness to IL-6. This effect could also be mimicked by C/EBP-β siRNA. In conclusion our study for the first time demonstrates C/EBP-β mediated transcriptional down regulation of DPP III by IL-6. Our results demonstrating negative correlation between IL-6 and DPP III taken together with previously reported prognostic significance of this cytokine in glioblastoma suggests that DPP III may prove useful as a prognostic marker.

Seth G.S. Medical College & KEM Hospital Oration

Microtubules, Small Plant Derived Molecules, and Uncontrolled Cancer Cell Division

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Microtubules are biological polymers of tubulin protein that make many intracellular machineries, the most notable being the mitotic and meiotic spindles responsible for cell division. Several plant derived toxins, such as colchicine and podophyllotoxin, block polymerization of these dynamic polymers and the cell division. Besides the highly dynamic arrays of dividing cells, microtubules also form relatively less dynamic arrays in post-mitotic cells such as neurons where they maintain axonal transport and axon integrity. The neuronal, gastrointestinal and immunological toxicities of colchicine and podophylotoxin make these compounds of limited use as anti-cancer drugs. Using a chemical-genetic approach, we have screened structural variants of colchicine and podophyllotoxin that just alter the dynamics of microtubule polymerization and depolymerization cycles without blocking the net polymerization in post mitotic cells with a hope to find small mitosis-arresting molecules without systemic toxicity. Our screens led to opium derived non-narcotic molecule, noscapine, that binds tubulin and mitigates microtubule-dynamics enough to halt progression of cell cycle. Owing to their weak cell cycle checkpoints, many cancer cells suffer gain or loss of multiple chromosomes during cell division when treated with noscapine and these cells self-destruct via apoptosis. Normal healthy cells with intact checkpoints remain arrested in mitosis for long periods of time long enough for noscapine to be metabolized and excreted from animal and human bodies. Thus noscapine and its further rationally designed derivatives (collectively called, noscapinoids) show significant anti-cancer activity against various cancer types. They are orally available safe drugs with favorable pharmacokinetics and are under Phase I/II clinical trials. I shall discuss the progress and future prospects of this line of research in novel chemotherapeutic intervention of disease.

S01

Confirmatory Follow-Up Testing for Inborn Errors of Metabolism

Rajesh Sharma

Inborn errors of metabolism (IEM) having a slow progressive course are not detectable clinically at birth and may result in severe neurologic damage and, in some cases, death before clinical diagnosis and treatment. A positive newborn screening result does not mean that the newborn definitely suffers from a disorder but it does mean that further diagnostic testing is needed. This lecture will focus on various inborn errors of metabolism, including organic acidurias, amino acidopathies, and fatty acid oxidation defects. Both clinical features and diagnostic testing will be discussed.

Learning Objectives:

1. Distinguish likely inborn errors of organic acid, amino acid, and fatty acid metabolism based on presenting signs and symptoms.
2. Become familiar with several of the more common metabolic disorders.
3. Identify appropriate testing for these types of disorders.

S02

Genetic Basis of Thalassemia Syndromes: Implications to Reduce Disease Burden

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Thalassemia syndromes constitute the commonest monogenic disorders in Indians and inheritance is autosomal recessive. Conservative estimates are >10,000 new cases/year of thalassemia major (TM) are born in India and this poses a considerable burden to the health care system. Parents of TM are carriers (beta thalassemia trait; βTT) and they constitute the asymptomatic reservoir of the disease. Identifying the spectrum of beta mutations in different regions has shown considerable heterogeneity. Approximately 70 beta mutations have been found in Indians but 6–7 mutations constitute 80–90%. Though IVS 1–5 G→C is the commonest mutation encountered, the frequency varies from 15 to 88% in different states. Fr 41/42 (-CTTT) is also widely encountered but commoner in the north. Increasing the awareness amongst the doctors, health professionals and general public will go a long way in reducing the prevalence of TM by widespread antenatal screening programs and cascade screening. Majority of the carriers can be identified by analyzing the automated blood cell counter reports. Hypochromic microcytosis (↓MCV and MCH) and relative erythrocytosis (↑ RBC counts) are found in βTT's. Increased HbA2 fraction of >4% on HPLC quantitation is the diagnostic hallmark of identifying carriers. Prompt screening of the husbands from the antenatal clinics and offering prenatal diagnosis should be done. For mutational characterization, ARMS-PCR is most widely applied followed by RDB analysis. Occasionally with rare mutations beta gene sequencing is done. All prenatal centres should perform chorionic villous biopsy/amniocentesis. Maternal contamination should be excluded using VNTR or STR analysis. Presently about 10 centres are offering prenatal diagnosis. Constant training programs to increase the manpower to perform screening and prenatal diagnosis are the need of the hour. Involvement of the State and Central Government is required to combat this potentially preventable genetic disorder on an urgent basis.

S03

Screening & Diagnosis of Inborn Errors of Metabolism-Pitfalls & Fallacies

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Inborn errors of metabolism (IEM) are considered as "individually rare but collectively not so rare", with the result large number of IEM's remain undetected. Strong suspicion of IEM can be considered if an infant shows lethargy, poor feeding, seizures, hypotonia, and excessive cry. Most acutely sick infants are treated empirically for sepsis whereas IEM may be the cause. We have come across 10 cases of MSUD, where the sibling death was attributed to sepsis. Early diagnosis of IEM by various laboratory tests is essential for timely management in order to prevent lethal irreversible complications. The unexpected and "mysterious" deterioration of a child after normal initial period is the most important signal of the presence of IEM. Knowledge of algorithm is very important inorder to test with right test at right time with right sample. At an advanced stage of illness, respiratory acidosis, severe hyperlactacidemia and secondary hyperammonemia can disturb the primary biological pattern, especially in disorders with rapid fatal course. Main problem faced is which diagnostic technology to use- Tandem mass spectrometry or GC-MS. Algorithm along with pitfalls and fallacies in interpretation of test results will be presented.

S04

Congenital Erythropoietic Porphyria with Erythrodontia: Genotype Phenotype Correlation in Two Indian Cases

A. K. Harith, Dr. Sandeep Arora, Dr. Seema Kapoor

Congenital Erythropoietic Porphyria is a rare inborn error of Heme biosynthesis characterized by photodermatitis and anaemia, erythrodontia and passage of reddish colored urine. One of the characteristic feature of these cases include the presence of bright pink fluorescence of the sclera, teeth and urine when observed under the Wood's lamp. Most of the diagnosis is made on clinical grounds and mutation analysis is rarely done. We present two cases of non related children who presented with features of photodermatitis, erythrodontia and anaemia.

Case 1: A thirteen years old child born out of consanguineous marriage was brought the the dermatology OPD with complaints of photodermatitis and anaemia. He had erythrodontia and anaemia which was hypochromic and microcytic in nature. Diagnosis of CEP was made on clinical grounds and the DNA of the proband and his parents were sent for mutation analysis. The child was found to be homozygous for c.A 56 G of Exon 2, (p.Y19C). Both the parents were carrier of this mutation.

Case 2: An eight year old child presented to the dermatology OPD with features of hypertrichosis on the face associated with hyperpigmentation and scars on the hands and feet. There was history of passing reddish urine since 2 years of the age. He had erythrodontia. He was found to be homozygous for c.C 710 T in Exon 10, (p.L237P). The mother of the child was a carrier of the mutation. As the child had lost his father it was not possible to study the mutation in the father.

Although both the mutations have been detected before, this is the first time that such mutations were detected in Indian population. The children are being managed symptomatically and being worked up for Bone Marrow Transplant.

S05

Role of Biomarkers in Prenatal and Newborn Screening of Genetic Disorder

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Biomarkers are cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids. In order to reduce, or even eliminate unnecessary invasive procedures, screening tests based on reliable serum biomarkers having efficient sensitivity and specificity has been developed successfully for various disorders worldwide.

Prenatal screening aims to estimate a woman's risk of having an affected fetus based on a combination of ultrasonographic soft markers and the concentration of certain biomarkers. Prenatal screening program based on biomarkers, PAPP-A, free hCG-β, AFP, and uE3 levels in first two trimesters of pregnancy is running successfully at Genetic Centre, GMCH-32, since 2007 for chromosomal aneuploidies and neural tube defects. More than 29,330 pregnant women from the area in & around Chandigarh have benefited till date through this screening program. The Reference range of free hCG-β, AFP and uE3 levels in second trimester of pregnancy have been successfully established in our population through triple test screening. The screening has helped in detection of 13 pregnancies affected with Down' Syndrome, more than 16 with Neural Tube Defects, 41 spontaneous miscarriages, 28 cases of intrauterine diseases, 40 cases of congenital malformations and several others pregnancy complications.

For newborn screening (NBS) we are using TSH, 17-OHP and glucose-6-phosphate dehydrogenase biomarkers for screening of congenital hypothyroidism, congenital adrenal hyperplasia and glucose-6-phosphate dehydrogenase deficiency respectively. Through NBS program till February 2015 we have screened 28,678 out 31,874 of born at GMCH-32. Incidence of Congenital hypothyroidism is 1:2600, G6PD Deficiency is 1:84 and congenital adrenal hyperplasia is 1:14,000 in our study.

S06

Challenges in TB Pathogen Research: From Basic Discovery to Control Strategies

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Tuberculosis (TB) is an enormous public health challenge worldwide. Approximately one-third of the world's population harbors latent TB infection (LTBI) and this enormous reservoir of latent infection greatly complicates efforts aimed at TB control. The success of *Mycobacterium tuberculosis* (Mtb) is attributed in significant measure to its ability to survive indefinitely in a dormant state within the host. During LTBI, tubercle bacilli survive indefinitely in a non-replicating dormant state that is refractory to anti-tubercular therapy. Therefore a priority research goal is to understand the properties of dormant bacteria in order to devise more effective strategies for TB control and to prevent reactivation and clinical disease.

DevR, a response regulator belonging to the DevR-DevS two-component system of Mtb, was discovered by subtractive RNA hybridization in my laboratory. It induces the expression of ~48 bacterial genes (DevR dormancy regulon) in response to hypoxia and other signals. The activity and function of DevR was characterized by various approaches, and on this basis, strategies to block signal transduction by DevR-DevS system were developed.

Another challenging area in the control of TB is the development of efficient, rapid, low cost, resource friendly diagnostic tests. There is considerable scope for the improvement of the smear microscopy test itself and for the development of improved diagnostic tests. In my laboratory, the detection of TB antigens has proved to be useful for the efficient diagnosis of pediatric tuberculous meningitis. Our experience with this approach for TB diagnosis will be discussed.

S07

Simple Diagnostic Test for TB: Challenges and Possibilities

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The last decade has seen the roll-out of several new WHO endorsed tests for diagnosis of TB and drug resistance. Several companies are focusing on the development of new molecular tests that meet or exceed the performance characteristics of GeneXpert but are cheaper and easier to implement. Simultaneously, operational studies provide evidence that only molecular tests that are highly sensitive and specific are unlikely to make a significant impact on patient morbidity and mortality. In recognition of the limitations of bacteriological confirmation, the WHO has identified the urgent need for biomarker-detection based tests for screening for TB, diagnosis of TB and of drug resistance. Unfortunately, few labs or companies have

moved towards research or product development for biomarker-based TB tests and the pipeline for such tests is weak. The complexity of the TB spectrum and the various manifestations of TB make it likely that TB control will require multiple comprehensive and cohesive tools. There is an urgent need for clear definition of the potential approaches that can lead to simple tests that can be implemented in TB-endemic countries, and focused efforts in rational development of these tests.

S08

Approach of Reverse Vaccinology in HIV-1 Infection

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Subtype C viruses cause >50% of HIV-1 infections worldwide and are majorly predominant in Indian patients infected with HIV-1. Analysis of human monoclonal antibodies (mAbs) developed from HIV-1 infected donors have enormously contributed to the identification of neutralization sensitive epitopes on the HIV-1 envelope glycoprotein. Dissecting the antibody specificities in the plasma of HIV-1 infected individuals that develop broadly neutralizing antibodies (bNAbs) is likely to provide useful information for refining target epitopes for vaccine design. The third variable region (V3) is a crucial target on gp120, primarily due to its involvement in co-receptor (CXCR4 or CCR5) binding and presence of epitopes recognized by bNAbs. So far, only a few human mAbs have been isolated from subtype-C infected individuals. We have isolated 3 mAbs # 277, 903 and 904 by hybridoma technology from HIV-1 seropositive drug naive patients, whose plasma antibodies exhibited good viral neutralization potential. The mAbs were selected from EBV transformed cultures with conformationally constrained Cholera-toxin-B containing V3C (V3C-CTB) fusion protein.

Further, using the EBV transformed cells of one patient, we constructed a recombinant phage library of anti-V3 single chain variable fragments (scFvs). We tested the mAbs and scFvs for their binding with HIV-1 derived proteins and peptides by ELISA and for neutralization against HIV-1 viruses by TZM-bl assays. The ELISA binding revealed a subtype-C and subtype-A specific binding of antibody 277 and 903 while 904 exhibited cross reactivity also with subtype-B V3. Epitope mapping of mAbs with overlapping V3 peptides showed exclusive binding to V3 crown. The antibodies displayed high and low neutralizing activity against 2/5 tier 1 and 1/6 tier 2 viruses respectively. The anti -V3 scFvs showed cross-reactivity against both the clade C and B V3 peptides and exhibited neutralization of a limited number of tier 1 viruses of clades C and B. Overall, we observed a resistance of the tier 2 viruses to neutralization by the anti-V3 mAbs and scFvs, despite exposure of the epitopes recognized by these antibodies on the native viruses. Our study suggests that the anti-V3 antibodies and scFvs derived from subtype-C infected Indian patients display neutralization potential against tier 1 viruses. Defining the epitope specificities of these mAbs and further experimental manipulations will be helpful in identification of epitopes, unique to clade C or shared with non-clade C viruses, for immunogen design.

One of the most studied regions on the HIV-1 envelope is the CD4bs, because of its highly conserved nature as the site responsible for binding to the CD4 receptor on target cells. Recently, we characterized the plasma of a cohort of HIV-1 infected Indian donors for the presence of CD4bs antibodies and their contribution to virus neutralization in. The identification of CD4bs dependent neutralizing antibodies in an HIV-1 infected Indian donor is a salient finding of this study and is supportive of ongoing efforts to induce similar antibodies by immunization.

S09

HIV-1/IVDU and Heart-Lung Interactions: Two Hits Worse Than One

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Combined antiretroviral therapy has clearly been effective in increasing the life-span of human immuno-deficiency virus (HIV-1) infected individuals and also in curbing the HIV-1 associated infectious complications. Nevertheless this extended life span has resulted in increased incidence of non-infectious complications including cardiovascular complications such as HIV-related pulmonary arterial hypertension (HRPAH). HIV-infection is now considered as one of the major causes of pulmonary hypertension worldwide with intravenous drug use (IVDU) identified as one of the most common risk factors in these individuals. Our findings consistently suggest augmentation of pulmonary vascular dysfunction in HIV infected IVDUs (mainly opioids +/- cocaine abusers) compared to HIV-infected non-drug users or un-infected IVDUs. Various studies from our lab using simian immuno-deficiency virus -infected macaques and cell culture model systems indicate synergistic or additive effect of opioids or cocaine on HIV-mediated pulmonary smooth muscle and endothelial dysfunction. Our recent findings using non-infectious HIV-transgenic rat model further supports an additive effect of cocaine and HIV on pulmonary vascular remodeling with elevated mean pulmonary arterial pressure and right ventricular systolic pressure in these rats on exposure to cocaine. In conclusion, drugs of abuse potentiate the damaging effect of HIV-proteins on pulmonary vasculature leading to higher incidence of HRPAH in HIV-infected IVDUs.

S10

Bio-Films of *Aspergillus Fumigatus*, Virulence, Pathogenicity and Novel Drug Targets

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Aspergillus fumigatus (*A. fumigatus)* is a well adapted opportunistic fungus that causes a severe and commonly fatal disease, invasive pulmonary aspergillosis (IPA) in highly immunocompromised patients, aspergilloma in patients with lung cavities and allergic bronchopulmonary aspergillosis (ABPA) in hypersensitive individuals. Recent studies have suggested that biofilm formation by *A. fumigatus* may be one of the most important virulence factors in IPA and aspergilloma. It is an arduous task to deal with the biofilm phenotyope of the fungus as it is refractory to most of the conventional antifungal treatment options. Several fungal constituents may contribute to the formation its biofilm structures on the host cells, including the cell wall components, the secondary metabolites and the

drug transporters. A variety of host components including surfactant proteins and serum pattern recognition molecules are also activated during aspergillosis which affect *the vivo* colonization by *A. fumigatus*. An in-depth analysis and understanding of both pathogen and host factors involved in the formation of *A. fumigatus* biofilms is necessary to devise newer and better antifungal targets for treating complex *A. fumigatus* biofilm associated diseases.

S11

Insulin Like Growth Factor (IGF-1) Assay and Analytical Changes

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Insulin-like growth factor 1 (IGF1), a 70 amino acid peptide hormone is the principal mediator of effects of growth hormone (GH). Since GH secretion is pulsatile in nature and is affected by many factors including sleep, feeding and exercise it is not a reliable marker for diagnosis of GH related disorders. On the other hand, IGF1 levels do not undergo short-term fluctuations in the manner that GH does making it the preferred IGF1 biomarker for the diagnosis of growth related disorders. There are several immunoassays available for IGF1 determination. Since majority (> 90%) of IGF1 circulates as a ternary complex bound to its principal carrier/binding protein, IGF binding protein 3 (IGFBP3) and acid labile subunit (ALS), the assay methodology used to quantitate IGF1 has to dissociate IGF1 from IGFBPs prior to quantitation. IGFBPs are known to be a source of interference in immunoassays and many techniques have been employed to circumvent this issue. Immunoassays rely on antibody specificity towards IGF1 and differential cross reactivity towards IGFBPs. Mass spectrometry (MS) has also been employed for quantitation of IGF1. Liquid chromatography tandem mass spectrometry (LC-MS/MS) assays for IGF1 rely on generating tryptic peptides followed by selective reaction monitoring (SRM) while LC high resolution accurate-mass mass spectrometry (LC-HRAMS) approaches for intact IGF1 rely on mass accuracy for reliable, robust and accurate quantitation. This review article will focus on the clinical assays available and the clinical utility of quantitative assessment of IGF1. IGF1 quantitation using diverse assay platforms including immunoassay, LC-MS/MS and LC-HRAMS are discussed in detail.

S12

Global Approaches to Medical Error Disclosure: A Quality Perspective in Diagnostic Laboratory Services

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In any health care process, adverse events resulting from errors are inevitable. Disclosure of an adverse event is an important element in managing the consequences of a medical error. Failure to inform the patient of adverse events caused by a medical error compromises the autonomy of the patient, as they are unable to properly consider and consent to proposed medical decisions that may be in their best interests. It also jeopardizes the opportunity to improve the quality in health care. We have previously reported a non-punitive, "no-fault" model for reporting medical errors. In order to analyze the progress made in the area of medical error disclosure and to understand the rationale for effective error disclosure policies, we reviewed and evaluated various error disclosure initiatives across Canada and other parts of the world (Australia, New Zealand and United States of America). The majority of provincial regulatory bodies in Canada have adopted some form of disclosure policy. However, these Canadian provincial initiatives remain isolated because of their non-obligatory nature and absence of federal or provincial laws on disclosure. In Australia, disclosure policy integrates the disclosure process with risk management analysis towards investigating the critical events. In New Zealand, in any adverse event, patients are rehabilitated and compensated through a no-fault state funded compensation scheme. This disclosure model supports the health care providers and strengthens the policy of honest disclosure. The United States Joint Commission on Accreditation of Healthcare Organizations mandated an open disclosure of any critical event during care to the patient or their families. By following an open disclosure policy, patient's autonomy can be preserved and malpractice claims can be reduced effectively. The complexities of medical error disclosure to patients present ideal opportunities for medical educators to probe how learners are balancing the ethical complexities involved in error disclosure. Effective communication between health care providers, patients and their families throughout the disclosure process is integral in sustaining and developing the physician patient relationship. We suggest that a uniform policy centered on addressing errors in a non-punitive manner and respecting the patient's right to an honest disclosure be a standard of care.

S13

A Comparative Study of HBA1C of Normal and Hemoglobinopathy Patients in D10 and Cobas Integra 400

Shyamali Pal

JMD Diagnostics Private Limited, Kolkata

HbA1c may be estimated by both ion-exchange HPLC & latex enhanced turbidimetric Immunoassay (Tina- quant). But HbA1c cannot be detected by D-10 in β-thalassemia and sickle cell anaemia. In such situation fructosamine estimation is still the best method of detection. The reasons can be –

(a) Formation of adult hemoglobin is so less that glycated hemoglobin remains below the linearity limit.
(b) b)HbF concentration is more than 16.5%, hence elute in the LA1c/cHb or A1c window.

In Tina-quant the binding of anti HbA1c is site specific, so small quantity is also detectable. When the result is below the linearity range the system information provides the delta absorbance(∂A) of HbA and HbA1c and result may be calculated.

Tina-quant assay may be performed at random and less expensive. So, more user friendly than HPLC.

As HPLC is more popularly being used so a comparative study of HbA1c by two systems was performed with patients having normal and variant hemoglobin.

120 patients with normal adult hemoglobin were tested in D10 HPLC system and the results compared in Cobas Integra 400 plus by Tina quant method.

The laboratory received 21 Hb-E trait, 36 β-thalassemia trait, 25 β-thalassemia homozygous, 5 Hb-E-β-thalassemia and 5 Hb-S trait patients. Hb variant chromatograms were obtained from D10 system.

HbA1c results were compared with Tina-quant.

The data comparison of normal adult hemoglobin shown correlation coefficient(R) 0.988. R for Hb-E trait & β-thalassemia trait are 0.976& 0.971. So, the variant chromatogram data of two methods are also in accordance. HPLC could not report HbA1c in β-thalassemia, E-β-thalassemia and SS patients due to the presence of low HbA. But Tina-quant assay produced the result. The results compared with plasma glucose (random) and Estimated Average Glucose calculated from obtained HbA1c and found out to be satisfactory.

Tina – quant assay is more acceptable than HPLC performed b as HbA1c of patients with variant hemoglobin of all types can be measured.

- Test may be performed randomly by (20minutes/test)Tina- quant. Hence, more user friendly than HPLC. It is less expensive also
- In cases with very low HbA the lab may obtain ∂A from reaction information and calculate the result.

S14

The Role of Biochemistry Tests in the Critical Care Setting

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Abstract: A large number of patients in the critical care setting include patients with acute coronary syndromes, heart failure, acute respiratory distress syndrome, systemic inflammatory response syndrome and trauma. Along with history, physical examination, imaging and ECG, laboratory investigations play a vital role, useful for the clinicians in making an accurate diagnosis, prognostication and formulating strategies for therapy. Biochemistry tests involve simple procedures and along with the clinical context it can aid in appropriate triage and segregation of patients. The presentation will focus on reviews of examining oxygenation status with lactate; assessment of volume overload with natriuretic peptides; identifying chest pain with troponins/CKMB, inflammatory markers or arterial blood gas parameters; to rule-in or rule-out infections with procalcitonin. The talk will also cover a small aspect of fluid homeostasis which get deranged frequently in serious illnesses.

S15

"Lean-Six Sigma" Application in a Hospital Laboratory

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Our current state value stream maps identified opportunities to use Lean – Six Sigma strategies in our process flow. Therefore, we design the laboratory process flow according to DMAIC (Define, Measure, Analyze, Improve and Control) flow.

Our aim is to pursue Lean-Six sigma in a hospital laboratory, in order to improve sigma metrics of 24 routine Biochemistry parameters and turnaround time (TAT).

In the define phase, the tools we used are project charter, Critical to Quality. In the measure phase, we use data plots & patterns, process capability. Here we calculate d.p.m.o. (defects per million opportunities) and express the value as sigma rating. In the analyze phase, tools used are root cause analysis. In the improve phase, we use brainstorming, decision analysis matrix. In the control phase, the tools are control charts, audits. Lean-six sigma helped us in the elimination of non value added (NVA) steps and focusing on the value added (VA) steps

We have seen that Out of 24 routine biochemistry parameters (ALP, ALT, Amylase, AST, Direct Bilirubin, Total Bilirubin, Calcium, HDL, Total Cholesterol, LDL, Albumin, Creatinine, CPK, GGT, Glucose, Iron, LDH, Lipase, Magnesium, Total Protein, Phosphorus, Triglycerides, UIBC, Urea), we have achieved Six Sigma for 18 parameters.

After receiving the STAT sample in the section, both the NVA times and VA times were around 45 minutes. So we eliminated NVA steps and our current TAT came down to 45 minutes from 1.5 hrs. For STAT test turn around time, in August, 2010, we have 74.7% compliance and 3.14 Sigma. In November, 2012 it has improved to 99.3% compliance and 5.63 Sigma.

Lean-Six sigma ensures that accurate and precise results are reported in a clinically relevant turn around time.

S16

Current Trends in Handling Post - Examination Phase in Clinical Laboratories

Dr. A. S. Kanagasabapathy

Formerly Prof.& Head of Clinical Biochemistry, CMC Vellore

International standards on post - examination phase:

CAP

All steps in the overall lab process between completion of analytical phase and receipt by physician. Examples are accuracy of data transmission across electronic interfaces, reflex testing, TAT and interpretability of reports

ISO 15189

Process following the examination including systematic review, formatting and interpretation, authorization for release, reporting and transmission of results and storage samples of the examinations

Post - examination phase activities:

Phase	Activity
Post - analytical *(activities within the lab)*	Report validation
	Feeding into LIS
	Communicating the results to clinician
	Reporting "alert/panic/critical" value
Post - post analytical *(activities outside the lab)*	Handing over the report (receipt of the Report by the physician)
	Interpretation by physician & follow up

ISO 15189:2012 # 5.8.2: Report attributes:

(a) comments on sample quality that might compromise examination results
(b) comments regarding sample suitability with respect to acceptance/rejection criteria
(c) critical results, where applicable
(d) comments on results, where applicable

Post examination phase – Common errors:

- Wrong validation
- Results that are delayed –exceeding assured TAT
- Results not reported
- Results reported to the wrong provider
- Incorrect results reported due to post-analytical data entry errors
- Transcription errors
- Problem in reporting critical values

Management of critical values:

- Compile the list in agreement with the users of its services
- Have procedures to identify critical values
- Specify the mode of transmission to the clinicians
- Specify who should be communicated and by whom
- Maintain records of notification of critical values
- Develop Quality Indicators to monitor lab performance

Post-examination Phase Quality Indicators:

- Result reporting accuracy
- Adequacy of information for interpretation of laboratory tests
- Result interpretation
- Report delivery turnaround time
- Consistency of critical values reporting
- Clinician's (patient's) satisfaction with laboratory services

IFCC Working group recommendations on post-analytical quality specifications will be presented

Parameters often included in auto-verification rules:

- Analyte reference ranges
- Instrument flags (short sample, possible bubble or clot, etc.)
- Indices (hemolysis, lipemia, icterus)
- QC flags
- Delta checks
- Relex testing
- Conditions for re-analysis

Let us encourage clinicians to feel free to contact lab directors and/or lab staff with questions about any lab test:

– interpretation of results
– assurance of quality of a test
– discussion of potential sources of error or variables to be considered in test interpretation

and the need for specialized tests

S17

Quality in Clinical Laboratories - How to Develop?

Neeraj Jain

Lab Director, Jain Diagnostics

Good quality is never brought about by accident; it is almost always the cumulative result of sincere intentions, dedicated effort, intelligent direction and skilful execution. As a choice, good quality may not necessarily be the easiest or the cheapest; however it is definitely the wisest for both patient health and welfare as well as laboratory credibility.

A well-defined quality system is must for ensuring quality. It has the following 10 key elements:

- Organization and management
- Personnel
- Laboratory instruments and equipment
- Procurement and external services
- Process control
- Document control
- Quality Control
- Control of non-conformity
- Internal audit
- Continual quality improvement

There is a cost associated with Quality. Quality costs can be offset by quality payoffs like enhanced reputation, loyal clientele, reduced system failures & machine downtime, less need for retesting for complaints etc. However there is no offset for medical implications that may be caused by poor quality.

Implementing an efficient Quality Management system does not guarantee a 100% error free laboratory, but it goes a long way in detecting errors that may occur commonly, and prevents them from recurring.

Thus, Quality is essential not just to all laboratories to provide accurate, reliable results, but it is essential to all aspects of healthcare and the medical profession.

S18

Impact of Altered Methionine Metabolism in the Pathogenesis of Alcoholic Liver Injury

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Alcoholic liver disease is a major health care problem worldwide. In our ongoing investigation on the mechanisms of ethanol-induced liver injury, our laboratory has previously shown that chronic ethanol exposure impairs several of the multiple steps in methionine metabolism. This results in decreasing the levels of the key methylating agent, hepatic S-adenosylmethionine, while increasing the levels of two toxic metabolites, homocysteine and S-adenosylhomocysteine thereby lowering the hepatocellular S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH) ratio. The ratio of SAM to SAH (also termed as the methylation potential) is an important metabolic indicator of cellular methylation status. This is because a decrease in the SAM:SAH ratio is associated with impaired activity of many of the 120 members of SAM-dependent methyltransferases resulting in the generation of many hallmark features of early alcoholic liver injury. We further showed that betaine administration can preserve the hepatic SAM:SAH ratio and thereby attenuates steatosis and other features of hepatic liver injury in ethanol-fed rodents. In expanding our findings to other organs and tissues of relevance to ALD, we found similar alcohol-induced changes in methionine metabolism occurring in the intestine as seen in the liver. We have data to show that alcohol-induced changes in intestinal SAM:SAH ratio disrupts tight junctions that preserve intestinal epithelial integrity. Through these impaired tight junctions, the viable bacteria and/or bacterial products from the gut lumen can translocate to the liver via the portal vein and trigger an inflammatory responsethat contributes to the progression of liver disease. We further show that betaine administration attenuates the ethanol-induced intestinal barrier dysfunction by preserving the distribution of tight junction proteins and promoting protective factors which mitigate the inflammatory response. These effects of betaine on intestinal epithelial integrity may contribute to the overall protective effect of betaine in attenuating ethanol-induced liver damage.

S19

Liver Function Tests: Clinical Interpretation

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Incidentally detected abnormality in liver function tests is a common situation encountered by physicians across all disciplines. It is import to remember that many of these patients do not have primary liver disease as most of the commonly performed markers are not specific for the liver, and are affected by a myriad of non-liver factors. Also, many of these tests like liver enzyme levels do not measure the function of the liver, but are markers of liver injury which is broadly of two types- hepatocellular and cholestatic. A combination of a careful history and clinical examination along with interpretation of pattern of liver test abnormalities can often identify type and etiology of liver disease, allowing for a targeted investigation approach. Severity of liver injury is best assessed by composite scores like the Model for End Stage Liver Disease rather than any single parameter.

S20

Type 2 Diabetes Mellitus is a Risk Factor for Progressive Liver Disease - A Study Among South Indian Population

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Non alcoholic fatty liver disease (NAFLD) has emerged as the most common cause chronic liver diseases in India and also worldwide; and is associated with elevated liver enzymes and modulates host immune function and biochemical variations. Type 2 Diabetes Mellitus poses an important risk factor for progressive liver in all aspects. Here in our study we investigated the role of anthropometric, biochemical and immunological parameters with NAFLD in the serum of 80 Diabetes Mellitus-2 subjects, 80 Diabetic patients with fatty liver and 80 diabetic patients without fatty liver attending the Medicine department. Ultrasound of the abdomen was done to detect fatty liver. Our study reveals that Diabetic patients with fatty liver had elevated SGPT/SGOT ratio, elevated oxidative stress parameters, high BMI and other anthropometric parameters and deranged lipid profile along with altered immunological parameters. A strong relationship exists between the hepatic immune response as well as alteration in the levels of different Oxidative stress parameters in Type-2 DM subjects with NAFLD. Obese individuals with increased waist hip ratio and BMI would act as a primary indicator in prediction of NAFLD in Type-2 DM patients along with LFT, and will be a handy tool for the clinicians for diagnosis and treatment.

S21

Cholesterol: A Focus in Atherosclerosis, Diabetes, Obesity & Carcinoma

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The insulin receptor (IR) and low-density lipoprotein receptor (LDLR) maintain glucose and lipid metabolism, respectively. Risk of cholesterol is found to be an intimate factor in developing carcinoma and atherosclerosis; eventually equally risk in long term events of diabetes and obesity.

To understand the potential interaction(s) between IR and LDLR in atherosclerotic, diabetes, obesity and carcinoma.

Primary and secondary antibodies, LDL/dil-LDL, radioisotopes, hormones, carcinogens, siRNA and subjects having diabetes, obesity and carcinoma were the primary components of this study. Immunoprecipitation, immunocytochemistry, western blot, confocal/electron microscopy, cellular uptake of LDL, and ultracentrifugation were the techniques used for the study.

Our study reveals that intracellular concentration of steroid is the prime regulator for maintaining LDLR expression. We have studied the possible role of potential interaction(s), the co-association, of IR & LDLR. The co-association makes LDLR functionally poor. Insulin disrupts the association between the two receptors and makes LDLR functionally more active. Co-existence of IR and LDLR promotes atherosclerosis in diabetes and obesity; more free and expressed LDLR is responsible to push cell cycle equilibrium towards over replication — eventually a lead to carcinogenesis.

The co-association of LDLR with IR and their dissociation by insulin may be an important part of the regulatory mechanism of the normal physiological receptor function in a biological system. Modulation of receptor co-association may be a potential therapeutic target to control cardiovascular and carcinogenic risk. Explore the co-association in more detail is our further goal in forthcoming future.

S22

Novel Biomarkers for Early Detection of Metabolic Syndrome

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Metabolic syndrome is a diagnosis that is not associated with one particular disease, but rather the probability of developing certain diseases or disorders as a result of a combination of defined risk factors. It is a designation for metabolic risk factors that, if occurring together, increase a person's risk for developing heart disease, stroke, and/or diabetes. An individual diagnosed with metabolic syndrome has an increased risk of diabetes that is five times greater than an individual without these metabolic factors and an increased risk of cardiovascular disease that is two times greater. In addition to cardiovascular disease, stroke, and diabetes, that are closely associated with metabolic syndrome, there are several complications and conditions that could also potentially occur. An individual with metabolic syndrome is susceptible to fatty liver disease, cholesterol gallstones, asthma, sleep apnea, osteoarthritis, pulmonary disease, renal disease, ocular complications, polycystic ovary syndrome, colon, endometrial, and breast cancers.

Diagnosis of metabolic syndrome does not necessarily correlate directly to a diagnosis of a specific disease. This diagnosis links several health risks with obesity and the other syndrome criteria. It is not a prediction of a short-term risk for cardiovascular disease and diabetes. Those with this syndrome have a high lifetime risk for these diseases. According to the American Heart Association, the risk factors for metabolic syndrome include: abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, insulin resistance or glucose intolerance, prothrombotic state, and pro-inflammatory state.

Since metabolic syndrome is a complex and multi-factorial disorder, there has been intensive search into the identification of novel biomarkers that are based on the pathophysiology underlying metabolic syndrome. The diagnostic criteria for metabolic syndrome require laboratory testing of glucose, triglycerides, and HDL-C. Several other analytes like LDL-C and hs-CRP may be monitored in those diagnosed with metabolic syndrome. Hyperinsulinemia occurs in insulin resistance and insulin levels can be quantitated in the laboratory. Fat cells regarded an endocrine organ which secretes a number of adipokines which are pro-inflammatory and pro-atherogenic and cause endothelial dysfunction. Some important pro-inflammatory, atherogenic, and antiatherogenic biomarkers like Tumor necrosis factor-alpha (TNF-alpha), Interleukin 6 (IL-6), Plasminogen activator inhibitor-1 (PAI-1), Adiponectin, Angiotensinogen, Leptin, visfatin, chemerin, resistin, ADMA, and MCP-1 play a central role in early diagnosis of metabolic syndrome. Though triglyceride measurement will continue to be simple screening test for the identification of high risk individuals, a combination of inflammatory, insulin resistance, and fat cell mass biomarkers may be used for more effective in early diagnosis of metabolic syndrome.

S23

Hypoxia Markers and Tumor Behavior in the Context of Human Gliomas

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It is well established that hypoxia is a natural corollary of the disordered autonomous growth of cancer cells, and the resulting neo-angiogenesis is an essential adaptive mechanism by cancer cells for their survival. Similarly, inflammation has been identified to be one of the tumour promoting 'enabling characteristics' of cancer cells. Hypoxia and inflammation interact with the process of carcinogenesis through a variety of signaling pathways, cytokines and transcription factors, leading to adverse effects on phenotype and patient survival. This also necessitates new pharmacological approaches to deal with hypoxic tumours. The above aspects will be discussed in the context of human glial tumours. An overview of molecular markers that can be added to histological classification will be discussed in the context of hypoxia related gene expression markers for possible therapeutic applications.

S24

Aluminum: Real Ghost in Aged Brains

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Aluminum (Al) is the third most abundant element in the earth crust and it is not essential for living organisms. This metal has no biological function; however, its exposure is very common to human beings by ways like industrialization, utensils, medicines, antiperspirants etc. It has been reported that as one ages Al accumulate in brain and other organs resulting in their dysfunction and toxicity. There is an increasing amount of evidence suggesting the involvement of Al^{+3} ions in a variety of neurodegenerative disorders such as Alzheimer's disease and Parkinsonism etc. Al could enter the brain from systemic circulation: blood-brain barrier (BBB), nasal-olfactory pathway and cerebrospinal fluid. The important carriers for brain Al influx may be Transferrin-mediated transport and monocarboxylate transporter. Our *in vivo* and *in vitro* results suggest the role of Al in brain dysfunction and neurodegenerative mechanism. We found Al exposure to be associated with oxidative stress, cognitive decline and ultrastructural changes in experimental animal's brain and the same results were found in our *in vitro* model system. Moreover, we also demonstrated the role of Al in mitochondrial and

endoplasmic reticulum stress mediated apoptotic pathway and found that Al induces ER stress and release of mitochondrial cytochrome c to promote apoptosis. Furthermore, in addition to its neurotoxicity, Al is a potent stimulator of the immune response. Our results also showed that Al activates inflammatory proteins and altered TNFα, IL1β, IL6 and IL10 mRNA levels. Overall, these results indicated that Al disturbed the redox state of the neuronal cells which results into the activation of various signaling including apoptosis and inflammatory pathways which promotes neuronal cell death.

S25

Central Role of Mitochondrial Dysfunction in Pathophysiology of Neurodegenerative Conditions: Modulation by Antioxidants and Mitochondrial Modulators

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Mitochondrial dysfunctions has for long been linked to the neuronal cell death in many neurodegenerative conditions like Huntington's disease, Parkinson's disease, etc. The therapeutic potential of combined administration of mitochondrial modulators: alpha-lipoic acid and acetyl-l-carnitine on mitochondrial dysfunctions in 3-NP-induced HD was evaluated. Our results reveal 3-NP administration resulted in compromise of mitochondrial functions in terms of: (1) impaired activity of mitochondrial respiratory chain enzymes, altered cytochrome levels, reduced histochemical staining of complex-II and IV, reduced in-gel activity of complex-I to V, and reduced mRNA expression of respiratory chain complexes; (2) enhanced mitochondrial oxidative stress indicated by increased malondialdehyde, protein carbonyls, reactive oxygen species and nitrite levels, along with decreased Mn-superoxide dismutase and catalase activity; (3) mitochondrial structural changes measured by mitochondrial swelling, reduced mitochondrial membrane potential and ultrastructure changes; (4) increased cytosolic cytochrome c levels, caspase-3 and -9 activity along with altered expression of apoptotic proteins (AIF, Bim, Bad, and Bax); and (5) impaired cognitive functions assessed using Morris water maze and Y-maze. Combination of mitochondrial modulators (alpha-lipoic acid + acetyl-l-carnitine) on the other hand ameliorated 3-NP-induced mitochondrial dysfunctions, oxidative stress, histologic alterations, and behavioural deficits, suggesting their therapeutic efficacy in the management of HD.

S26

Apolipoproteins as Risk Markers for CVD

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Dyslipidemias are known to associate with the risk and pathology of cardiovascular disorders. This notion along with observational evidences traditionally have recommended study of lipid profiles to assess the risk of cardiovascular disorders in a person or in a population. The metabolism of the endogenous and exogenous lipids is a function of the protein part of a lipoprotein, the apolipoproteins and, hence, it is logical that time to time the importance of apolipoproteins as cardiac risk factors had been envisioned and studies had been undertaken. Over the years we had been working in this area.

We enrolled 500 patients diagnosed for CAD by the cardiologists at AIIMS, New Delhi and equal number of controls from the NCR region. The demographic details of the study subjects were collected using a questionnaire. A well designed exclusion and inclusion criteria was followed for the recruitment of the study subjects.

Lipid and lipoprotein profiles were determined and their association with CAD were evaluated. Patients were sub-grouped as those presenting with NCAD, SVD, DVD and TVD. The lipid, lipoprotein and selected apolipo-protein profiles were studied and their association with CAD was evaluated.

Of the lipid and lipo-protein parameters, only HDL emerged as the independent risk factor of CAD. Levels of apo A1 were lower in patients compared to controls, correlated with the severity of CAD. Our results suggested that the levels of A1, are genetically determined. In recent years, we took up a study on para-oxonase an HDL associated enzyme to evaluate its potential as a risk marker of CAD.. The studies had been carried out both at the protein and gene level. Lower levels of arylesterase associated with the risk of CAD.

To conclude, while low HDL is an independent risk factor of CAD for the Asian Indians, levels of apo A1 may also be helpful in identifying the people at the risk of developing severe CAD. Association of paraoxonase with CAD needs further confirmation.

I express my heartfelt gratitude to Prof. LM Srivstava who initiated me in this area of research. The contribution of my students especially Dr. Shivani Chhabra and Dr. Imtiaz Ahmed is fondly acknowledged.

S27

Role of microRNAs in Cardiovascular Complications of Diabetes

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Cardiovascular complications account for significant morbidity and mortality in diabetic population. Epidemiological and clinical trial data have also confirmed greater incidence and prevalence of heart failure in diabetes. Cardiomyocyte hypertrophy and myocardial fibrosis are the established pathological features of the diabetic heart and are associated with dysregulation of gene expression of key pathway genes. The search for the basic mechanisms that are responsible for the development and progression of diabetic cardiomyopathy has been exhaustive; nonetheless, no single unifying mechanism has been uncovered that explains the development and progression of heart failure in diabetic patients. Central dogma of molecular biology states that expression of protein coding genes can be regulated at various levels from transcription to translation. In the past, most studies have focused on protein coding genes and their regulation at the transcriptional level. Recent studies have uncovered a potentially important role for a family of tiny noncoding regulatory RNAs, known as microRNAs (miRNAs or miRs), in the transcriptional and post transcriptional regulation of gene expression. Further, microRNAs have been reported to regulate diverse aspects of cardiac function and also play an important role in the pathogenesis of heart failure through their ability to regulate the expression levels of genes

that govern the process of adaptive and maladaptive cardiac remodeling. However, our understanding of the role that microRNAs play in heart failure is limited. An overview of putative role of micro RNAs targeting various pathways involved in the pathogenesis of cardiovascular complications in diabetes milieu will be elucidated.

S28

miRNAs: A Novel Therapeutic & Diagnostic Tool Against Cardiovascular Diseases

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MicroRNAs are class of short ($\sim$18–24 nucleotides) endogenous non-coding RNAs that post-transcriptionaly regulate gene expression. Distinct miRNA signatures have been observed in cardiovascular diseases, including arrhythmias (miR-1, miR-133 and miR-208a), fibrosis (miR-21and miR-29), cardiac remodelling (miR-208 and miR-133) etc. Similarly miR-21, miR-155, and miR-126 have been implicated in vascular diseases. Since microRNA profiling in animal models of cardiovascular disease and in human biopsies has revealed signature patterns of miRNAs diagnostic for numerous cardiovascular disorders. A comprehensive miRNA profiling was carried out to generate a miRNA signature for third most common cardiovascular disease –thrombosis. We started with system biology approach to identify potential miRNAs involved in pathogenesis of this disease. Among potential candidate miRNAs, an inverse correlation with thrombus formation was observed. Restoration of miRNAs in thrombotic animals via *in vivo* delivery of the miR mimic resulted in a significant reduction in thrombus size, without disturbing hemostasis. Interestingly, we also found reduced miRNA levels clinically confirmed venous thrombosis patients when compared to control. Our translational study identifies a novel mechanism involving miRNA in regulating thrombus formation and widens the scope for miRNAs to be used as a therapeutic option against cardiovascular diseases.

S29

Evaluation of Stable and Unstable Vulnerable Atherosclerotic Plaques Requires Different Markers !

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Atherosclerosis is now widely accepted to be a multifactorial disease with a major influence of inflammatory factors. Local inflammation in the form of macrophages and other immune-competent cells may be responsible for weakening of the fibrous cap ultimately leading to a acute coronary event like a myocardial Infarction or stroke. This clinical event is usually of sudden onset though the underlying process of Atherosclerosis takes decades to develop. However, till date it is not possible to predict or give risk evaluation of such a event. A large lipid core and a thin fibrous cap is the usual site of a rupture.

In out studies of stable ischemic plaques, we have evaluated 400 cases and controls who were selected for the study on basis of severity of obstruction (> or <50% occlusion of coronary arteries). Serum CETP, LCAT, TNF-α, ox LDL, Paraoxanase and SOD levels were measured by ELISA. Serum Apo A1 and Apo B levels were measured on Synchron CX-9 by immunoturbidimetric method. Genetic polymorphism for CETP-629C/A and TNF 308G/A were studied by PCR followed by RFLP using restriction enzymes AvaI and Nco1 respectively.

Oxidative stress markers (Paraoxanase, ox LDL, SOD, and GPX) were found to be significantly elevated in cases having >50% obstruction of coronary arteries ($p < 0.000$, $p = 0.025$, $p < 0.000$, $p < 0.000$). Inflammatory markers (TNF-α, NF-κB and IL-6) were also significantly elevated in cases ($p = 0.000$, $p < 0.05$, and $p = 0.003$ respectively). In the lipid markers, LCAT was not significantly associated with atherosclerosis ($p = 0.764$) whereas CETP was associated with a p value of 0.035. Among the genetic alleles studied, no intergenotypic variation of 308-G/A promoter region polymorphism of the TNF-α gene was seen but CETP alleles showed homozygous AA genotype was more in cases with increased (> 50%) coronary artery blockage. For stable atherosclerotic plaques it is the markers like Paroxonase and SOD which have implications of increasing plaque size : further risk stratification is possible by evaluating inflammatory markers like TNF- α. However, none of these markers imply plaque vulnerability.

In case of a acute coronary event like myocardial infarction, markers like MMP-9 which are responsible for vascular re-modelling, were found to be significantly elevated in patients who had a myocardial infarction within 24 hrs of sample collection. Besides imaging studies and calcium scoring of plaques, thrombotic and tissue markers are also of use. Our study on MCP-1 and MMP-9 have demonstrated elevations in acute coronary events.

Patients having stable atherosclerotic plaques and vulnerable unstable plaques need to be evaluated by separate markers as their clinical course and acute events are different and it is not possible to predict an acute coronary event on the basis of conventional markers.

S30

One-Carbon Metabolism and Response to Methionine Load in Healthy Young Women

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K.E.M.Hospital Research Centre, Pune, India, has been involved in maternal nutrition studies. In 1999 we reported that Indian children are small at birth but big at 8 yrs. We also reported that maternal nutrition before and during pregnancy determines foetal size and composition. In 2001 we reported that maternal intake of micronutrients (green leafy vegetables, dairy products and fruits) and erythrocyte folate concentrations predict new born size and offspring adiposity. In 2003 our report was that plasma vitamin B12 and folate concentrations during pregnancy are associated with insulin resistance in the offspring. In 2005, we reported higher plasma homocysteine concentrations at 28 weeks of gestation predicts smaller offspring size in rural India, In 2007, the report was that the new born size and childhood growth is associated with cardio vascular disease risk factors at 6 yrs. of age. These informations obtained in our unit point to the critical role of one carbon metabolism and development and its sensitivity to nutritional and environmental influences affecting the fetus which impacts fetal growth and 'programming' of the

metabolism of infant causing LBW. Low birth weight (LBW) is a critical problem and a major contributor to the morbidity and mortality. A strong association has been shown in adulthood between LBW and diabetes mellitus, hypertension and coronary heart disease.

Data from the Pune Maternal Nutrition Study show that a relationship exists between maternal folate levels and the birth weight of the infant as well as show a correlation between maternal vitamin B12 and folate status and insulin resistance in their children at 6 yrs of age.

The findings of physiological measurements of methionine metabolism by administering methionine load allow us to identify the effect of subclinical changes in nutrient status on one-carbon metabolism of the mother will be discussed. The relation of the physiological data to fetal growth as assessed by birth weight and body composition with metabolic pathways involved will be discussed during the allotted time.

S31

Influence of Iron Status on Iodine Utilization and Thyroid Function in the Hilly and the Plain Regions of Eastern Nepal

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Iodine and iron deficiency presents a major public health problem in Nepal with 19.4% of its 27 million people being at risk of iodine deficiency and 48% of total school children are suffering from iron deficiency anemia. Iron deficiency mainly manifested in the form of iron deficiency anemia is due to lack of adequate iron and vitamins in diet and hook worm infestation, though other factors may also cause it. Iodine deficiency is mainly attributed to low levels of iodine content in the diet.

Aim of the present study was to explore the effect of iron status in utilization of Iodine and thyroid function in hilly and plain regions of Eastern Nepal.

A community based cross section study was conducted among 489 primary school children (52.14% boys and 47.86.0% girls) from Bhojpur (Hill) and Jhapa (Plain) districts of Easten Nepal. Hemoglobin, Iron, TIBC and thyroid function parameters (fT_3, fT_4 and TSH) were estimated by commercial test kits. UIC and SIC were measured by APDM and RTK method respectively. Data were expressed in frequency, percentage, Mean ± SD and median (IQR) according to the nature data. Chi-square test, Independent't' test, Pearsons and Spearman's correlation and Mann-Whitney U test were applied to test the significance considering $p \leq 0.05$ at 95% confidence interval.

The Median UIC of Bhojpur was significantly lower as compared to Jhapa (147.35 (83.6, 261.85) μg/L vs 363.31 (219.04, 481.5) μg/L, $p = 0.001$). Iron status parameters: Iron and TIBC were significantly different between SAC of Jhapa and Bhojpur district (Iron; 98.33 ± 43.86 μg/dL vs 74.32 ± 32.0 μg/L, $p = 0.0001$, TIBC: 260.60 ± 76.23 μg/dL vs 376.65 ± 41.92 μg/dL, $p = 0.0001$) but hemoglobin was not significantly different (13.62 ± 1.62 g/dL vs 13.10 ± 1.43 g/dL, $p = 0.052$). Thyroid function parameters fT_3 and fT_4 were significantly different in SAC of Jhapa and Bhojpur (fT_3; 3.07 ± 0.56 pg/mL vs 2.90 ± 0.50 pg/mL $p = 0.050$, fT_4; 1.14 ± 0.18 ng/dL vs 1.37 ± 0.23 ng/dL, $p = 0.0001$), but TSH was not significantly different. TIBC was significantly negatively correlated with, fT_3 and UIC ($r = -0.182$, $p = 0.007$ and $r = -0.208$, $p = 0.002$).

Thus, iron status has influence at the functional level of iodine rather than its nutritional level.

S32

Serum PONase Activity in Relation to Lipid Profile and PON1 Gene Variants in Age Related Macular Degeneration

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Age related Macular Degeneration (AMD), a chronic disease of the central retina is considered multifactorial. The atherogenic pathophysiology seen in AMD involves altered lipid metabolism, though there are conflicting results. Paraoxonase 1 (PON1), a HDL-associated esterase, is known to possess anti-oxidant and anti-atherogenic properties. This study focuses on the expression and activities of PON as well as the genotype / phenotype correlation in AMD cases of South Indian population.

In this prospective case-control study, a total of 50 patients diagnosed with AMD (Mean age: 68 ± 19y) and 30 unrelated healthy controls were recruited and 30 healthy controls (Mean age: 53 ± 24y). The serum oxLDL and Plasma Homocysteine levels were estimated by ELISA. Serum PON activities were estimated by spectrophotometry. Serum lipid profile was done in clinical analyzer. PON gene expression by qPCR and protein by WB, IF and FACS analysis. SNPs coding for Q192R and L55 M variants by PCR based restricted fragment length polymorphism (RFLP) method.

Serum total Cholesterol (TC), Triglycerides (TG), oxLDL, paraoxonase (PONase), arylesterase (AREase) and thiolactonase (PON-HCTLase) activities were significantly elevated in AMD patients than controls. Stepwise regression analysis showed that there is a significant and positive correlation between the high density lipoprotein (HDL) levels and the activities of PONase and PON-HCTLase in AMD patients ($p = 0.015$ & $p < 0.001$ respectively). PON1 protein in serum and PON2 in PBMC, both at protein and gene expression level were significantly increased in PBMC of AMD patients. In AMD patients with PON (Q192R) polymorphism, a significant increase in the serum TC, TG and PONase activity was observed for the GA and AA genotypes compared to controls, while LDL was increased in GA genotype. In PON1 (L55 M) polymorphism analysis, serum TC and TG levels were significantly increased in AMD patients with AA and AT genotypes. oxLDL and LDL were increased only in AT genotype.

The significant increase of lipid parameters in AMD patients and it genetic association clearly demonstrates derangement of lipid metabolism in AMD.

S33

Grape (*Vitis vinifera*) Extracts Protect against Radiation-Induced Oxidative Stress and DNA Damage

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Ionizing radiation (IR) causes oxidative stress through the overwhelming generation of reactive oxygen species (ROS) in the living cells leading further to the oxidative damage to biomolecules. Grapes (*Vitis vinifera*) contain several bioactive phytochemicals and are the richest source of antioxidant. In this study, we investigated and compared *in vitro* antioxidant activity and DNA damage protective property of the grape extracts of four different cultivars, including the Thompson seedless, Flame seedless, Kishmish chorni and Red globe. The activities of ascorbic acid oxidase and catalase significantly ($p < 0.01$) differed among extracts within the same cultivar, while that of peroxidase and polyphenol oxidase did not differ significantly among extracts of any cultivar. *In vitro* antioxidant activities were assessed by ferric-reducing antioxidant power (FRAP) assay and ABTS. The superoxide radical-scavenging activity was higher in the seed as compared to the skin or pulp of the same cultivar. DNA damage was evaluated in acellular system using pBR322 plasmid relaxation, as well in genomic DNA from blood. Grape extract was able to effectively scavenge free radicals in vitro. It could significantly prevent radiation-induced DNA damage. Furthermore, the protective action of grape depends on the source of extract and type of the cultivars.

S34

Glutathione Degradation: New Pathways, New Insights

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Glutathione is essential metabolite for the growth of all eukaryotes, from yeasts to man, barring a few amitochondrial protozoans. It plays a key role in maintaining redox homeostasis in living cells, and disruption of this homeostasis is observed in many disease conditions. Glutathione homeostasis is controlled and determined by different processes. One of the key processes in glutathione homeostasis is glutathione degradation. Since 1970, the only enzyme known to be responsible for glutathione degradation has been γ-glutamyl transpeptidase, an enzyme of the γ-glutamyl cycle of glutathione metabolism proposed by Meister.

Genetic studies with the yeast *Saccharomyces cerevisiae,* have led to the discovery of several new pathways of glutathione degradation. One of these pathways, the Dug pathway, involves a multimeric complex that is unique to yeasts and fungi. The second pathway, the ChaC1 pathway involves a protein previously known to be a pro-apoptic protein induced by ER stress and is present from *E. coli* to man. Knock down of the ChaC1 pathway affects development in the mouse and zebra fish. A third constitutive pathway for glutathione degradation present in higher eukaryotes has also now been identified. These pathways, their discovery, the structure of the ChaC enzyme, and the implications in human disease would be presented.

S35

Development of Cyclometalated Iridium Complexes as Agents of Photoinduced Cell Death Caused by Oxygen Free Radicals

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Photodynamic therapy (PDT) is a promising anticancer therapeutic technique which is less invasive, more targeted, and effective in the treatment of many of the superficial cancers like skin and oral cancers and of other inflammatory conditions such as psoriasis and rheumatoid arthritis. Most of the superficial cancers are caused due to the exposure to inflammatory stimuli like UV and use of various carcinogenic substances such as tobacco. Development of inexpensive and targeted methods of treatment of these cancers is important for a country like India where the exposure to these carcinogenic stimuli is very common in daily life. Photodynamic therapy uses a photosensitizer which can be taken up by cancerous tissue and on photoirradiation generates oxygen free radicals which cause the destruction of the cells. Thus, there is a selective ablation of cancerous cells using PDT which uses a photosensitizing compound combined with photoirradiation, resulting in the production of oxygen free radicals. We have developed a series of heteroleptic phosphorescent cyclometalated iridium(III) complexes among which one (C2) showed specific localization in the endoplasmic reticulum of human breast carcinoma cells and caused efficient photoinduced cell death when illuminated by light at 405 nm. Cells treated with C2 and exposed to photoirradiation showed progressive membrane blebbing, contraction of cells and generation of cellular processes, leading to nearly complete cell death after 1 h of exposure. On photoinduction, C2 was found to generate oxygen free radicals in the cells, as demonstrated by DCFDA staining, which led to membrane damage and ultimately apoptotic cell death. C2 also showed very minimal autonomous cytotoxicity, suggesting it to be an effective candidate as a photosensitizing agent. Together, these data show C2, and related iridium(III) complexes, as highly efficient potential agents for development as photosensitizers for the photodynamic therapy of cancer.

S36

Does Oxidative Stress Influences Carotid Artery Intima-Media Thickness: An Early Predictor of Coronary Artery Disease in High Risk Subjects

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We aimed to assess whether oxidative stress influences carotid Intima media thickness, which is an early predictor of coronary artery disease (CAD). To demonstrate this, we enrolled patients without and with CAD and healthy controls. Demographic details were obtained from all the subjects and ***carotid intimamedia thickness (CIMT) measured by*** high frequency ultrasound *and other parametrs* by spectrophotometric methods. The distribution of cardiovascular risk factors in patients without CAD and CAD cases were smokers (16% vs 56%), hypertension (26% vs 64%), diabetes (16% vs 56%) and dyslipidemia (18% vs 58%) and positive family history (4% vs 38%). None of the control group had any cardiovascular risk factors. The CIMT, plasma homocyseine, and oxidative stress markers such as plasma malondialdehyde, protein carbonyls, were significantly elevated and glutathione and nitrite levels were significantly decreased in CAD cases as compared to cases without CAD and healthy controls. On the other hand, CIMT was significantly increased in cases without CAD like diabetes, dyslipidemia, hypertension and smoking as compared to healthy controls. However, the intensity of increase was lower than CAD cases. CIMT also increased with the severity of disease in CAD cases. Homocysteine and oxidative stress are positively correlated with CIMT. In patients with diabetes, CIMT increased as duration of diabetes increases and also in poorly controlled diabetes. In CAD group, when number of vessel involvement (severity of coronary disease) increases, the CIMT also increases. In conclusion, increase in oxidative stress, decrease of nitric oxide strongly influences the increase in CIMT, subsequently leading to endothelial dysfunction, an early indicator of CAD in high risk groups.

S37

One-Window Laboratory Investigations Transformation of the Discipline of Laboratory Science

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The science, art and commerce of the discipline of laboratory science has been undergoing a palpable transformation leading towards a goal where the patient has not to attend several laboratories for giving samples and later run from pillar to post to collect reports over different scales of time. One collection centre having automated phlebotomy tube labeler (APTL), post-phlebotomy automated sample sorter and conveyer belt, pre-analytical automation, help all samples correctly collected and to reach the central laboratory on time. This has narrowed down the employment cost and almost eliminated the human error from the process.

There is emergence of one-lab-concept consisting of in a single track automated hematology analyzer connected with pre-analytical automation in the upstream and in downstream with coagulation analyzer, clinical biochemistry analyzer, immunoassay analyzer and even automated ELISA washer and reader. Post-analytical automation has been dichotomized for generation of patient's reports through laboratory information system (LIS) and hospital information system (HIS) in one hand and preservation of patient's sample, if and as required, on short term and long time basis. Even the histopathological and cytological techniques, media preparation and microbial culture are put under partial or full automation.

What are left to manual modes are all kinds of microscopic skills in hematology, pathology and microbiology laboratories along with investigations like cytochemistry, histochemistry, immunocytochemistry and immunohistochemistry, other immunological investigations, nephelometry and spectrophotometry-based and flowcytometric investigations. Manual operations are still prevalent in most of the molecular techniques (PCR and its variants).

High performance liquid chromatography (HPLC), High-end molecular techniques, Next generation gene sequencer (NGS), Cell sorter, Confocal microscope, MALDI-TOF Mass Spectroscopy, Liquid chromatography- and Gas chromatography mass spectrometry (LC-MS and GC-MS), inductively coupled Plasma MS (ICP-MS), have become part of common research facility.

In this way there is development of a three-tier clinical and research laboratories each nested within the other with automation at the bottom, techniques those require human skill at the middle, and high-end research and developmental facility at the top. What is an imperative at this juncture is reorganization of the academics, teaching and research in the discipline of laboratory science.

There is an urgent requirement of (A) sensitization of the undergraduates towards this nested three–tier laboratory systems and (B) creation of a post graduate course (MD) in laboratory medicine which would encompass all of automated first tier and the easier functions of manual tier-two. This will ensure availability of laboratory professional with Four-in-One expertise. The difficult portions of the manual tier-two will continue to remain parts of respective discipline of Pathology, Microbiology and Biochemistry with respective MD course in their discipline, drawing nourishment both from the top (tier-III) and the roots (tier-I). It is envisaged that tier-I might become the basic postgraduate degree to learn laboratory science and existing tier II postgraduate courses are likely to be converted into DM courses in their respective specialty.

S38

Restructuring the Clinical Diagnostic Lab: One Lab Concept

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Modern day clinical diagnostic laboratory has been transformed by automation. Most of the routine/baseline investigations are now-a-days performed on largely automated platforms, be it clinical chemistry autoanalyzers, hematology cell counters, coagulation analyzers, immunoassays or to a certain extent rapid molecular diagnostics. Besides, with the advent of versatile instrumentations like Flowcytometers, qPCRs or HPLC it is high time the present day diagnostic laboratories are re-structured. But most importantly the evaluation of different reports emanating from a lab holistically leading to diagnosis should be the aim of any diagnostic service.

Modern day automated integrated platforms has not only reduced the turn-around-time but also created a scope for holistic approach in laboratory diagnosis. Hence it is crucial that we give the diagnostic lab a new shape in the following terms:

1. Redefining the scope of the laboratories: blurring of the boundaries between the disciplines like pathology, biochemistry and microbiology
2. Evaluation of patient reports holistically, thereby giving more meaning to it from a diagnostic point of view
3. Evolution of the modern day laboratory physician so as to enable him/her to cater to the emerging needs of automation and holistic interpretation of laboratory data.

The one-lab concept of a modern diagnostic lab rests on the categorization of services provided based on the degree of automation involved and the amount of expertise, both technical and medical, required for generation of quality reports. Ideally it should have a three tier structure, depending upon the scope of services provided by the lab and the available resources.

1. Tier I: Catering to routine/ baseline parameters, mostly on automated platforms requiring minimal technical intervention or medical experience.
2. Tier II: Catering to specialised services requiring specialised technical training and significant medical experience. Quality of reports would also depend upon clinical understanding and mutual cooperation between the lab and the clinician-counterparts.
3. Tier III: Catering to very specialized services with high end-equipments, sometimes bordering towards research. This may be a centralized facility for several hospitals or labs and concentrating mostly on rare diseases.

For the proper management of the Modern day lab we need technicians and laboratory physicians who understand the concepts of laboratory automation, are capable of maintaining a high degree of quality in the reports generated and also bringing in a holistic diagnostic approach to the laboratory sciences. They would enable the laboratory to interact with the treating physicians, understand their needs and thereby guide the diagnostic approach so as to bring down the overall cost and time involved in the process.

The one lab concept has the potential to revolutionize the field of laboratory diagnostics in the primary and secondary tier of the health care system and is the need of the day for a 'resources-constrained' country like ours.

S39

Role of Antioxidants in Changing of Lifestyle Diseases: A Frontier for Translational Research

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The objective of the study was to understand the association between metabolic components and antioxidants relationship in changing of lifestyle diseases

Oxygen plays an important role in metabolic management and acts like double–edged sword. It may be lifesaving or destructing. Trends are alarming toward various metabolic diseases such as obesity, hypertension, diabetes, insulin resistance, dyslipidemia, stroke, NAFLD, early menopause, depression, CVD, musculoskeletal disorders, inflammation, COPD etc. Insulin resistance, obesity becomes major public health problem worldwide. Insulin resistance altered the levels of glucose, lipid or methyl glyoxal which increased the advanced glycation (AGEs) end products which releases the cytokines. The pro-inflammatory cytokines are responsible for endothelial dysfunction, dysregulation of calcium channel and ultimately leads to hypertension, fatty liver, diabetes, coronary vascular disease. Similarly, oxidative stress generates the lipid radicals and activates to oxidize the LDL which allows recruiting more foam cells that damages the endothelial wall for atherosclerotic lesion.

Evidence suggest that various biomarkers such as adipokines, IR, BMI, proinflammatory and anti-inflammatory cytokine, endotoxins, AGEs, antioxidants, oxidants stress index, genetic polymorphism such as SNPs, mitochondrial dysfunction etc requires to manage with the intervention of antioxidants profile, omega 3 fatty acids, reduction of dietary fructose and probiotic. Multivariate analysis may provide the significant information with the association between the age, genders, waist circumference, hypertension, IR etc.

Changes in life style and some drug treatments for metabolic syndrome may provide the significant information for selection of appropriate antioxidants with an appropriate amount. Omics era helps to understand the metabolic pathogenesis and also for designing of DNA based personalized nutrition. Thus, the quality of life can be improved.

S40

Novel Molecular Targets of New Antifungals in *Aspergillus Fumigatus*

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Jhansi

Aspergillus induced diseases have increasingly been recognized as threat to public health. The successful cure of aspergillosis has been extremely difficult mainly because the diagnosis at early stage of infection is not possible and there is paucity of the effective and safe drugs. The A. fumigatus synthesizes a large number of molecules including antigens, toxins and other metabolites, many of which have not been identified yet, although information of their presence may exist in the recently sequenced genome. These molecules of A. fumigatus may be responsible for the development and progression of the disease. Earlier studies on proteome profiling of A. fumigatus revealed expression of hundreds of major protein molecules in the pathogen, however, precise functions were not assigned. The identification of some of these pathogenic protein molecules as targets for new antifungals by pharmacoproteomic tools may help in developing effective drugs for aspergillosis.

The proteome analysis of *A. fumigatus* after treatment with a new antifungal N,N,N-Triethyl-11-(4-methyl-2-oxo-2H-benzopyran-7-yloxy)-11-oxoundecan-1-aminium bromide (named SCD-1) showed that the compound exerted its effect on the pathogen by complete inhibition and the decrease in abundance of several key proteins. The SCD-1 completely inhibited the expression of four proteins of crucial metabolic processes and decreased the abundance of two proteins belonging to the pathogen-specific riboflavin synthesis pathway in A. fumigatus. Thus, these proteins could be considered as important molecular targets of SCD-1. There was an increased expression of the molecules belonging to compensatory pathways of the pathogen, so as to maintain the cellular equilibrium and counter the stress generated by SCD-1. However, this response also must have contributed to

cause death of pathogen by disturbing the metabolic equilibrium in the cell. Our observations set forth a panel of protein molecules of A. fumigatus that could be targeted to achieve lethal effect on the pathogen. This would be helpful in designing specific antifungals, including more effective derivatives of SCD-1, for treatment of Aspergillus-induced disorders.

S41

Migration and Stemness Characteristics of Glioma Cells in Hypoxia

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Glioma remains the most malignant primary brain tumor with poor prognosis despite advances in therapy. Hypoxia plays a central role in tumor progression driving induction of genes which work in concert with an aim to make tumor cells more aggressive. We have described the role of hypoxia in modulating migration (and hence metastasis) and stemness of glioma cells. We have demonstrated that a subset of U87MG human glioma cells with increased migratory potential exhibit an increase in migration inducing Mena transcripts along with an induction in expression of Sox-2 and Oct-4 when exposed to hypoxia. Mechanistically, we could identify a link among the three features driving tumor progression- hypoxia (HIF-2α), migration (Pan Mena, Mena INV) and stemness (Sox-2, Oct-4) by knockdown and overexpression strategies in U87MG and A172 glioma cells. This HIF-2α- Sox-2/Oct-4-Mena (INV) axis was strongly activated under hypoxia. Furthermore, we obtained significant positive correlation among these markers studied in human Glioblastoma Multiforme samples. Such an effect of hypoxia was common in other transformed cells and was not seen in Grade II glioma cells and normal astrocytes. The results obtained identify Mena INV as a potential migratory marker whose expression is found to be elevated in cancer cells exposed to hypoxia. Further, a new mechanistic link involving an upstream influence of HIF-2α on stemness and invasive markers is also proposed based on the results obtained.

S42

Old Protein New Tricks: Exploring Diphtheria Toxin Beyond Toxicity

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Diphtheria toxin (DT) is an exotoxin and has been widely studied to understand its structure and function. Heparin Binding EGF-like Growth Factor (HB-EGF) is the receptor for DT. DT binds to cell surface HB-EGF, through its receptor-binding domain. On binding, DT is internalized through receptor-mediated endocytosis. HB-EGF is overexpressed in several types of cancers, wounds and in atherosclerotic plaques. As it is expressed on cell surface, HB-EGF is a potent target for homing drugs to specific cells. We have cloned and expressed recombinant receptor-binding domain of DT. This protein is non-toxic but binds to cell surface HB-EGF. We have shown that recombinant receptor-binding domain of DT can home a drug to cells overexpressing HB-EGF. We further show that this protein can enhance uptake of nanoparticles to cells expressing HB-EGF. We have also designed and created peptides based on the receptor-binding domain of DT. Such peptides retain binding to the receptor. Our work shows that proteins and peptides derived from the receptor-binding domain of DT may find uses in cellular targeting, in blocking cell signaling and in assays to detect HB-EGF in clinical samples.

S43

Primary Culture of Cancer Cells Established from Ascites of Ovarian Cancer Patients: As a Tool for Patient Tailored Therapy

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Ovarian cancer is one of the most lethal gynecological malignancies with very poor 5 year survival rate. The initial symptoms are vague and often ignored by Indian women. As a result it is most often diagnosed in the third or fourth stage when surgery alone is not sufficient. All patients are empirically treated with platinum compounds and Taxol as first line chemotherapy. However, most patients are either resistant to these drugs or come back with relapse. They are then subjected to second line drugs which increase morbidity and mortality. We can reduce this morbidity if we are able to choose the best chemotherapeutic agent for individual patients based on the sensitivity of the ovarian cancer cells to an array of chemotherapeutic agents. In our laboratory we standardized a method to establish primary culture of ovarian cancer cells shed in ascitic fluid. We have shown in our previous studies that ovarian cancer cells of ascites reflects the molecular profile of the primary tumor. Therefore these primary cultures have immense potential to be used as a tool to detect drug resistance and design patient tailored therapy.

Ascitic fluid along with corresponding primary tumor tissue was collected from twenty untreated epithelial ovarian cancer patients. Ten primary cultures were established from ascites obtained from untreated ovarian cancer patients in MCDB 105 and M199 medium (ratio 1:1). Expression of p53 and survivin was evaluated by immunohistochemistry. Down regulation of survivin was done by siRNA and sensitivity to paclitaxel was evaluated by MTT assay. Grapes like cluster of ovarian cancer cells present in ascites attached and gave a characteristic cobble stone appearance. The expression of p53 and survivin was high in tumor and ascitic cells as compared to normal tissue. Treatment with Survivin siRNA resulted in a 6 fold decrease in Survivin expression in primary cultures. Survivin siRNA treatment significantly increased the sensitivity of the primary ovarian cancer cell cultures to paclitaxel.

The limitation in this method is that we achieved only a 50% success rate in establishing primary culture which limits its widespread application in clinical settings. A robust method of establishing primary cultures from ascites as well as tumor tissue needs to be developed. This will ensure successful establishment of culture from

all samples attempted and will enable us to offer patient tailored therapy to all ovarian cancer patients.

S44

Embryonic Stem Cells: From Basic to Translational Research

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Pluripotent stem cells (PSCs) can be of two different types a) Embryonic stem cells **(ESC) and induced pluripotent stem cells (iPSC)**. ESC as their name suggests, are derived from embryos, more precisely day 5-6 blastocysts in humans. iPSC can be generated directly from any adult cells by introducing four specific genes. The iPSC technology was pioneered by Shinya Yamanaka's lab in Kyoto, Japan in 2006 that fetched him the 2012 Nobel Prize along with Sir John Gurdon. Most unique about these pluripotent cells are that they are virtually immortal i.e. they undergo self-renewal and can give rise to all the types of specialized cells in the body. Scientific knowledge on PSCs is increasing rapidly, leading to exciting opportunities for development of PSC-based therapies for regenerative medicine as well as establishment of in vitro tests for drug discovery, preclinical safety pharmacology and toxicology. Moreover current directed differentiation protocols are able to produce high yields of cardiomyocytes from ESCs and studies in small animal models of cardiovascular disease have proven sustained engraftment and functional efficacy. In my talk, I am going to cover all these aspects with respect to ESCs.

S45

Cardiovascular Risk Markers; Current Status & Future Trends

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The importance of cardiovascular markers arises from the fact that cardiovascular disease remains a leading cause of death not only in India but worldwide. Many patients who have a cardiovascular event or are diagnosed with cardiovascular disease have normal, or at least not highly abnormal lipid levels, and traditional lipid screening protocols failed to identify many patients as high-risk. For these reasons, there has been an on-going effort to develop novel cardiovascular markers and risk markers. Unlike cardiovascular markers, risk marker testing is used to help estimate the risk for future disease e.g. PLAC test. Commonly measured risk markers in the clinical laboratory include apolipoprotein A1/apolipoprotein B100, Lp(a), oxidized LDL, LpPLA2, hsCRP, heart type fatty acid binding protein(Fab), lipoprotein particle size and concentration, the myocyte stress and injury biomarkers like BNP, soluable ST2 and TropI. The goal of this research is to uncover biomarkers that can be easily measured but will serve as reliable windows into the patient's overall cardiovascular health. Such markers not only used for diagnosis, but also used to identify which patients are at high-risk for adverse cardiovascular events before they have these events.

S46

Proteomic Analysis of Circulating Monocytes in Indian Women with Discordant Bone Mineral Density (BMD)

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Osteoclasts are bone resorbing cells and increase in their number leads to decrease in BMD. Circulating monocytes (MO) are precursors of osteoclast hence proteins relevant to osteoclast can be unravelled in MO which may have important implications in the pathogenesis of osteoporosis. Previous studies, in non-Caucasian population on MO proteomics were focused on either premenopausal or postmenopausal women. The aim of the study was to identify MO proteins that are differentially expressed in low versus high BMD condition common to premenopausal and postmenopausal in Indian women in a single iTRAQ based platform. Premenopausal women ($n = 100$, 30–40 years) and postmenopausal women participants ($n = 100$, 50–60 years) were enrolled and their femoral BMD was measured by DXA. Based on centile distributions of BMD, participants were categorized into premenopause low BMD, premenopause high BMD, postmenopause low BMD and postmenopause high BMD. Proteins were extracted from MO isolated from blood samples collected from 20 participants from each category and were subjected to quantitative proteomics.

Heat Shock Protein 27 (HSP27) was distinctly upregulated in low BMD condition in both pre and post categories. Intracellular ELISA confirmed that total HSP27 and phosphorylated HSP27 (pHSP27) were significantly elevated in low BMD condition in pre and post categories ($p < 0.05$). Serum levels of pHSP27 were significantly elevated in low BMD groups in premenopausal and postmenopausal categories ($p < 0.05$). pHSP27 exhibited a significant Odds Ratio (OR) to differentiate between low and high BMD in both premenopausal ($OR = 1.734$, $p = 0.013$) and postmenopausal ($OR = 1.463$, $p = 0.042$) categories.

This study highlights a novel relation between serum pHSP27 and BMD in Indian women.

S47

Biomarkers in Early Diagnosis of Carcinoma Breast

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Breast cancer is the third most common cancer in women accounting for highest morbidity and mortality worldwide. The increasing global burden emphasizes the need to understand the sequence of biochemical events taking place in tumor cells and to establish the role of certain biomarkers in early diagnosis. Tumor cells are known to produce Reactive Oxygen Species (ROS) at a greater pace than the non transformed cells, causing oxidative stress

leading to cell proliferation, increased inflammatory conditions and progression of the disease.

The present study was aimed to decipher the correlation of pro-oxidant and anti-oxidant imbalance with metabolic changes in enzyme activities and their importance in diagnosis and prognosis of breast carcinoma.

The study was conducted in the Department of Biochemistry in collaboration with Surgery Department, Sri Guru Nanak Dev Hospital Amritsar. 150 clinically diagnosed on the basis of mammography findings and histopathologically proven breast cancer patients were selected. Equal number of age matched healthy females was taken as controls. Informed consent was obtained. Blood samples of the cancer patients with and without metastasis (pre and post-operative) and that of the controls were analyzed for serum ADA (adenosine deaminase), LDH, SOD, 5' Nucleotidase and plasma GSH levels. Estimations were done with standard protocols.

The activity of ADA, 5' Nucleotidase, ALP, and LDH were significantly raised ($p < 0.001$) in female patients as compared to normal females. The activities of these diagnostic enzymes improved post-operatively. A clear increase was observed in these enzymes from stage 1 to stage 4 of breast carcinoma. However, LDH and ALP showed a drastic surge from stage 3 to stage 4 indicating their role in secondaries. Oxidative stress increased with the progression of the disease as shown by significantly low SOD activity and GSH levels in female patients. However, after mastectomy oxidative stress as well as enzyme activity declines.

Serum ADA is useful biomarker for the early diagnosis of breast carcinoma whereas LDH and ALP provide significant information regarding metastasis. In nutshell, this cluster of enzymes can help in supplementing the information provided by TNM system of classification and staging of breast cancer patients. This would further help in early diagnosis and adopting proper treatment strategy for the survival of the patient.

S48

Paroxysmal Nocturnal Hemoglobinuria (PNH) and Deficiency of GPI Anchored Proteins

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Paroxysmal nocturnal hemoglobinuria (PNH) is an uncommon, acquired, clonal hematopoietic stem cell disorder. It presents as chronic hemolytic anemia, classically associated with recurrent hemoglobinuria that may also show features of bone marrow failure (cytopenias) and vascular thrombosis. However many patients may present with chronic anemia or other manifestations without any definite history of paroxysmal hemoglobinuria, though evidence for intravascular hemolysis in the form of hemosiderinuria may be evident. PNH has been extensively studied and many of the mysteries relating to its pathogenesis have been unraveled in the last decade. Whereas convincing explanation for the thrombotic episodes is still lacking, intravascular hemolysis has been related to increased sensitivity of the RBCs to the hemolytic action of activated complement. This increased sensitivity was shown to result from the inability of erythrocytes to inactivate the complement on their surface due to the absence of specific proteins which are attached on the surface of cells. Absence or deficiency of glycosyl phosphatidyl inositol (GPI) anchor results in an abnormality to anchor these proteins to the cell surface, leading to deficiency of several proteins. GPI anchored protein deficiency is almost always due to somatic mutations in phosphatidylinositol glycan class A (PIGA), a gene involved in the first step of GPI anchor biosynthesis. Eculizumab, a first in-class monoclonal antibody that inhibits terminal complement, is the treatment of choice for patients with severe manifestations of PNH.

S49

Redox Regulation of Platelet Activation in Aging

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The incidence of thrombotic events such as stroke, myocardial infarction, deep vein thrombosis and pulmonary embolism increases with age. However, the mechanisms by which aging contributes to these phenotypes are not known. We have investigated the prothrombotic role of oxidative stress during aging specifically its effect on regulation of platelet activation. In a recent study, we tested the hypothesis that aged mice overexpressing the antioxidant enzyme glutathione peroxidase-1 (Gpx1) are protected from experimental thrombosis. The findings demonstrated that the time to stable occlusion after photochemical injury of the carotid artery, was significantly faster in aged mice compared with younger mice. Unlike wild-type mice, aged transgenic mice overexpressing Gpx1 (Gpx1 Tg) did not exhibit faster times to occlusion of the carotid artery. Aged wild-type mice also exhibited increased susceptibility to venous thrombosis post inferior vena cava ligation and Gpx1 Tg mice were protected from this aging-related enhanced susceptibility to venous thrombosis. Age-dependent platelet hyperactivation, evidenced by increased intra-platelets hydrogen peroxide, fibrinogen binding, and activation of fibrinogen receptor αIIbβ3, were observed in thrombin-activated platelets from wild-type but not Gpx1 Tg mice. Enhanced platelet activation responses in aged mice were also prevented by PEG-catalase or apocynin, an inhibitor of NADPH oxidase. Aged mice displayed increased intra-platelet expression of $p47^{phox}$ and superoxide dismutase-1, suggesting a mechanistic pathway for increased H_2O_2 generation. Further, the accumulation of platelet on collagen surface was found increased with platelets from aged wild type mice but not Gpx1 Tg mice. Consistent with the findings in mice, platelets from older human subjects also exhibited an increase in activation of αIIbβ3 and increased accumulation of platelets on collagen surface compared to platelets from younger individuals. In summary, aged mice develop increased susceptibility to both arterial and venous thrombosis, and that H_2O_2-mediated platelet hyper activation is a likely mechanism leading to this prothrombotic phenotype. A similar mechanism may also lead to activation of platelets in aged humans. These findings suggest that therapeutic strategies targeted toward lowering platelet H_2O_2 levels may have the potential to decrease thrombotic complications of aging. One potential strategy would be to target NADPH oxidase, since several small molecule and peptide-based inhibitors of Nox2-containing NADPH oxidases subunits are currently in development.

S50

A Rare Case of Drug Induced Methemoglobinemia

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Breach Candy Hospital Trust

Methemoglobin is formed when ferrous ion (fe++) in the heme group is oxdised to ferric (fe+++) state. Methemoglobin is unable to combine with oxygen resulting in decreased oxygen carrying capacity of the blood. Reference range of methemoglobin is <1 %. However when its levels increase >15%, it results in pseudocyanosis. Levels >30% can cause headache and dyspnea and levels >70% can also prove to be fatal. Methemoglobinemia can be inherited or acquired from drugs or chemicals containing nitro or amino group and raised levels can be treated by intravenous methylene blue or red cell transfusion.

A 70 Years old male was admitted to the hospital with ulcers in the perianal region, and was treated with PRILOX cream (lignocaine 2%) for a period of nearly 3 weeks. The patient was admitted with low oxygen saturation and dyspnea. The whole blood was arterial but dark in colour and hence analysed for methemoglobin by co-oximetry on ABL 800 SERIES blood gas analyzer. Co oximetry revealed methemoglobin levels of 5.7%. The patient was treated with high flow oxygen and the lignocaine drug was withdrawn. Methemoglobin levels were normalized, which improved the oxygen saturation.

Thus, raised Methemoglobin levels can be the hidden reason for the decreased oxygen saturation and need immediated attention.

S51

Mutational Landscape of Gallbladder Cancer

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Gallbladder carcinoma (GBC) is a common gastrointestinal malignancy with specific geographical and ethnic variation. North India has one of the highest incidences of GBC in the world. High throughput approaches such as microarray and next generation sequencing have significantly advanced our understanding of mutation landscape in common cancers. However, mutational profile of GBC remains largely ambiguous. Here, we have performed somatic mutation and copy number profiling of GBC using targeted sequencing through NGS platform and Oncoscan microarray.

We performed targeted sequencing of all exons of 409 cancer-related genes of 12 well characterized tumor samples from GBC cases matched with blood samples on Ion Torrent proton platform using AmpliSeq comprehensive cancer panel. These 409 genes encompass over 50% of the Wellcome Trust Sanger Institute cancer gene census. For microarray studies, we included 18 histopathologically confirmed Gallbladder cancer cases. The study material was formalin fixed paraffin embedded blocks of the patients.

We used Ion reporter, an ion torrent specific data analysis pipeline, to identify somatic mutations. The identified somatic alterations include deleterious single nucleotide variants in KRAS, CDKN2A, MIR548F1 etc. and novel somatic variants in CREBBP, MYH9, CDK12 etc. By microarray studies, we detected substantial number of recurrent CNA. Most recurrent gains were at chromosome 12q (53%), chromosome 20p (69%) and most recurrent loss were at chromosome position chromosome 4q (46%). The common genes affected in copy number loss process are NPP6, IRF2, CASP3, CCDC111, PRIMPOL, MLF1IP, CENPU, ACSL1, ACSL1, SLED1 on chromosome 4, whereas genes involved in copy number gain process is HMGA2, RPSAP52, in chromosome 12.14.3, RASSF3 gene on chromosome 12 14.2, and MACROD2-IT1, MACROD2, MACROD2 on chromosome 20. Currently, we are validating somatic mutation and copy number variations. Further, this study will involve pathway analysis and clinical correlations.

S52

Targeting Increased Copper Levels in Diethylnitrosamine Induced Hepatocellular Carcinoma Cells in Rats by Epigallocatechin-3-gallate (EGCG)

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We have hypothesized that mobilization of endogenous copper ions by plant polyphenols such as EGCG and consequent oxidative degradation of cellular DNA could be an important mechanism of their anticancer properties.

Over the years we have validated our hypothesis to a considerable degree. We further confirm the hypothesis by using cells derived from hepatocellular carcinoma (HCC) induced in rats by diethylnitrosamine (DEN). Comet assay was used to examine EGCG mediated oxidative breakage of cellular DNA in HCC cells. We induced HCC in rats by DEN using a protocol standardised in our lab.

We show that in such carcinoma cells, there is a progressive elevation in copper levels at various intervals after DEN administration. Concurrently with increasing copper levels, EGCG mediated DNA breakage in malignant cells is also increased. The cell membrane permeable copper chelator neocuproine inhibited the EGCG-mediated cellular DNA degradation, whereas the membrane impermeable chelator bathocuproine was ineffective. Iron and zinc specific chelators were also ineffective. In summary, we provide evidence of copper accumulation in HCC. We further show that cellular DNA in such cells is preferentially targeted by EGCG. Copper is a major metal ion present in nuclei and it is well established that tissue, cellular and serum copper levels are considerably elevated in various malignancies. Such a common mechanism would better explain the anticancer effects of polyphenols with diverse chemical structures as also the preferential cytotoxicity towards cancer cells.

S53

Exploiting Cell Cycle by Noscapinoids: Non-toxic Preclinical Cancer Chemotherapy

Harish C. Joshi

Noscapinoids are a novel class of tubulin-binding agents with anticancer activity against human xenografts of drug-resistant lymphomas and hormone-refractory breast cancers in nude mice. They suppress microtubule dynamics at concentrations that do not

alter the total polymer mass of tubulin and decrease the otherwise normal rapid transition frequencies between growth and shortening phases while significantly increasing the percentage of time microtubules spend in an idle 'pause' state. Our work demonstrates that noscapinoids briefly arrest cell cycle progression at the G2/M phase by formation of multipolar spindles that either succumbs directly to apoptosis or after an abortive mitotic exit to an abnormal G1-like state. We have confirmed apoptosis by externalization of phosphatidylserine on the plasma membrane and emergence of a massive cell population containing sub-G1 DNA amounts. Noscapinoid therapy successfully regresses several human neoplasms implanted in immunodeficient mice. Unlike currently-available antimitotics (such as *taxanes* and *vincas*), noscapinoids are non-toxic to tissues with frequently dividing cells including the spleen and gut. Furthermore, noscapinoid therapy is non-immunosuppressive as seen by unaltered counts of CD4+, CD8+, B220+ and NK1.1+ cells. Most importantly, they do not show neurotoxicity quantifiable by neuropathological, electrophysiological, and behavioral measurements. Our data thus provide compelling evidence for the clinical evaluation of noscapinoid therapy for cancer management.

S54

Deciphering the Biochemical Pathway(s) by Clinical Target Specific Novel Anticancer Inhibitor

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Cancer is a diverse class of diseases which differs widely in their cause and biology. The aberrant behavior of cancer is generally reflected by the up-regulation of certain oncogenic signaling pathways that promote proliferation, inhibit apoptosis and enable the cancer to metastasize and evoke angiogenesis. Due to earlier failures and challenges, the most of the current cancer drug development work has shifted towards the molecular targeted therapies, conceived primarily from the better understanding of the molecular pathology of the cancer origins and epidemiological data. So, number of components of various genetic and epigenetic signaling cascade have been the subject of cancer researchers for successful outcome. Among these, designing the PI3 K inhibitors has formed an attractive avenue for cancer chemotherapeutics and a key aspect which raises much excitement is, its potential importance in regulating the various processes of cell development and survival during cancer initiation, prognosis or drug resistance. The emphasis of the talk is to put forth our recent findings, wherein we identified a novel inhibitor against PI3 K pathway and elucidated its signaling inhibition in various preclinical cancer models for the possible anticancer therapeutics.

S55

Role of p53 Deletion in T-lymphomagenesis

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p53 has been referred to as the guardian of the genome, and its loss is pathogenetic in several types of cancer. Our study tries to unravel the developmental defects and oncogenesis subsequent to p53 loss in the hematopoietic system.

Hematopoietic cell specific p53 deletion was achieved by crossing floxed p53 mice with a Vav1-Cre mouse. Tumors were analyzed by flowcytometry and histopathology. Gene expression was studied by qPCR, RNA-Seq and protein levels by Western blotting.

Initially pan-hematopoietic p53 deficiency led to relative myeloid and T-cell hyperplasia in the peripheral blood which progressed to frank malignancies. Virtually all the tumors that developed before 6 months of age were thymic or splenic T-cell lymphomas. Older mice developed myeloid or mixed malignancies. FACS analysis of the T-cell tumors showed a mixture of tumors, including double-negative (CD4-CD8-; DN), double-positive (CD4+ CD8+), or single positive for either CD4 or CD8. Even prior to the development of tumors, p53-/- mice showed accelerated T-cell development, with very few cells in the DN1-3 stages of differentiation in the thymus. Hence T-cell neoplasms likely derived from a cell that has passed beta-selection in an abnormal fashion. These T-cell tumors showed Notch1 activation and this activation was multifactorial. We found increased Mdm2, increased active GSK-3β and decreased Numb levels, all of which are expected to contribute to increased Notch1 activity. In addition, the majority of the tumors showed a dramatic change in the RNA isoform of Ikaros, which is known to be a repressor of Notch1 target genes. Murine thymocytes from wild type and p53 -/- mice were subsetted into various fractions (DN1, DN2, DN3, DN4, DP, CD4 and CD8) and the transcriptome was studied by RNA-seq. We found clusters of genes with differential expression patterns between the two sets of mice. These are the core p53 dependent genes and are likely to be involved in the lymphomagenesis observed in these mice.

Hematopoietic p53 deletion led to Notch1 dependent T-cell acute lymphoblastic leukemia (T-ALL) in young mice. Notch1 is a central regulator of T-cell development in humans and is deregulated in human T-ALL. Subsequently, the transcriptome regulated by p53 in thymic subsets was uncovered by RNA-Seq. The differentially regulated pathways thus identified can be utilized as novel diagnostic and therapeutic targets in T-ALL in the future.

S56

Unraveling the Link Between HLA-B27 Misfolding and Ankylosing Spondylitis

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Several hypotheses have been proposed to explain the strong association of HLA-B27 with a group of inflammatory arthritic disorders, known as the spondyloarthropathies. According to one such theory, HLA-B27 participates in disease pathogenesis through its enhanced ability to form cysteine-mediated heavy chain-dimer populations. However, none of the theories provide any scope to explain the differential disease association of the different subtypes of HLA-B27. We have hypothesized a detailed novel molecular mechanism based on phenomenological arguments supported by molecular modeling and molecular dynamics simulations for the misfolding of B27 chains, resulting in formation of high molecular weight (HMW) aggregates, in the absence of beta-2 m and peptide. According to this hypothesis, a helix-unfolding transition allows a part of the B27 chain (identical in sequence to a known B27 ligand) to become free to loop

around and bind to a structurally distorted peptide-binding cleft, in the same B27 molecule (auto-display) or in a neighboring molecule (cross-auto display). We have studied such HMW forms of B27, through biophysical and biochemical investigations, and further, using various cysteine mutants of B27, we have been able to investigate the nature of misfolded B27 in the disease associated (B2705) vs the non-disease-associated (B2709) subtypes, to obtain some direct, rather than circumstantial evidence, both, for cross-auto display and the role of such display in pathogenesis.

S57

Profiling the Membrane Proteome of Aminoglycosides Resistant *Mycobacterium tuberculosis* Isolates

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Aminoglycosides, amikacin (AK) and kanamycin (KM) are second line anti-tuberculosis drugs used to treat tuberculosis (TB) and resistance to them affects the treatment. Identification and characterization of membrane or membrane associated proteins of *Mycobacterium tuberculosis* is important due to their anticipated role in virulence and bacterial-host interactions. These proteins are likely to function as enzymes, receptors, transporters or signal transducers that could be of vital importance to the microbe and hence could qualify as drug targets. We compared membrane and membrane associated proteins of AK and KM resistant and susceptible *M. tuberculosis* isolates by 2DE coupled with MALDI-TOF/TOF-MS and bioinformatic tools. Twelve proteins were found to have increased intensities in resistant isolates. Among these three are proteins with unknown functions. Docking showed that both drugs bind to the conserved domain of these hypothetical proteins and GPS-PUP predicted potential pupylation sites within them. Increased intensities of these proteins and proteasome subunit alpha might not only be to neutralize/modulate the drug molecules but also involved in protein turnover to overcome the AK and KM resistance. Further research is needed to explore how these potential protein targets contribute to resistance of AK and KM. Such information could be helpful for the development of newer diagnostics and therapeutic agents for better treatment particularly drug resistance.

S58

Calcium, Beta-2-Microglobulin and the Disease Known as Dialysis-Related Amyloidosis (Associated with Treatment for Kidney Disease): Unexpected Connections

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We have recently discovered (serendipitiously) that the protein beta-2-microglobulin which is present in blood - and which is nearly impossible to aggregate and precipitate at neutral pH, at serum concentrations - readily forms micro-aggregates in the presence of serum concentrations of calcium. Such aggregates then grow in size and undergo precipitation into larger amorphous aggregates when there is even a mild increase in the concentration of either calcium, or beta-2-m. Initially, such aggregates can be dissolved through the addition of EDTA. However, with time, amorphous aggregates become amyloid-like in molecular character as well as morphology over a time period of a few weeks. Our observations offer an explanation for why hemodialysis invariably results in dialysis-related amyloidosis (DRA), since microaggregates of beta-2-m are unlikely to cross the dialysis membrane, resulting in a heightened concentration of the protein on one side of the membrane. Our observations also suggest that EDTA treatment during dialysis might ameliorate DRA, by preventing calcium-mediated hyper-aggregation. Importantly, these observations also suggest that excessive calcium supplementation might be harmful.

S59

Exploration of Autoantigens by Proteomic Analysis from Patients of Autoimmune Diseases

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Autoimmune disease is a condition triggered by targeting of self-molecules due to the deterioration of immunological tolerance. It is initiated by the cross-reactivity between host and pathogenic proteins, which can be attributed to molecular mimicry. A wide range of autoimmune diseases cause notable mortality and morbidity worldwide. We have identified new autoantigens, present in autoimmune diseases like rheumatic heart disease (RHD) and autoimmune uveitis (AU). In both the diseases, initially the autoantigens were recognized by cross-reactivity with patient serum in Western blotting. Unique peptides identified in mitral valve tissues of RHD patients were further characterized through LC-MS. Additionally, the proteomic data was also confirmed by real time PCR. Similarly, 2D-electrophoresis of vitreous fluid revealed the auto-reactive peptides in AU patients compared to infectious uveitis and finally characterized by MALDI-TOF MS/MS. Further in-vivo studies are in the process to validate our identified autoantigens in order to develop therapeutic strategy.

S60

The Challenges and Promises of Cancer Stem Cells in Solid Tumors: Role of Proteomics

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Recent evidences on cancer biology suggest that a subset of cells called cancer stem cells are present within the tumor mass which possess tumorigenic capacity. These are the cells responsible

for propagation, relapse and metastatic dissemination. These cells have certain stem cell-like properties, e.g. quiescence, self-renewal, asymmetric division, and multidrug resistance which allow them to drive tumor growth and evade conventional therapies. The idea that many cancers are organized as hierarchies sustained by cancer stem cells (CSCs) at their apex has generated a lot of excitement in many quarters of the cancer research community. From our studies, we concluded that a small portion of cells in the tumors may have characteristics of CSC and these cells could be an attractive source for CSC research. However, very little is known about the regulation of cancer stem cell (CSC) maintenance pathways in cancer and how these are affected by cancer-specific genetic alterations and by treatment. Proteomics is emerging as a powerful tool to identify the signaling complexes and pathways that control multi- and pluri-potency and stem cell differentiation. Proteome based data reflect the functional activities of encoded proteins and enhance knowledge about deregulation of pathways as a result of altered expression and activities of proteins in CSC. The best way to study the CSC proteome is probably to capture them in their normal microenvironment, that is, from within the patient tumor. This requires markers that can discriminate between CSCs and non-CSCs. Proteomics workflows may employ different combinations of fractionation methods. A number of markers and assays have been designed to isolate and characterize the cancer stem cell population from the bulk tumor and based on these we have identified CSC population in a number of tumors.

Cancer treatment may be improved by specifically targeting the key biologic pathways that are critical to the activity and survival of cancer stem cells. The commonly used chemotherapeutic agents show limited efficacy against the CSC and there is a need to develop new therapeutic modalities. The development of CSC-targeted treatments face a number of potential hurdles, including normal stem cell toxicity and the acquisition of treatment resistance, which must be considered in order to maximize the chance that such therapies will be successful. Various approaches have been suggested to target the CSCs which may help in increasing the overall disease free survival and recurrence cancer.

In the absence of surrogate clinical markers that adequately reflect the biology of the disease, survival should remain a primary endpoint of therapeutic efficacy when studying new treatments.

S61

Current Scenario of Prevalence of Vitamin D Deficiency in Ostensibly Healthy Indian Population: A Hospital Based Retrospective Study

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25-hydroxy vitamin D (25(OH) vit D) deficiency is an important public health problem, particularly in the Indian sub-continent. The objective of the present study was to study the prevalence of 25(OH) vit D in different age groups. 25(OH) vit D assays of 26,346 ostensibly healthy individuals enrolled under executive health checkup at Medanta The Medicity, Gurgaon, over a period of 3 years were extracted from the hospital information system and reviewed extensively. 25(OH) vit D deficiency was defined as 25(OH) vit D < 20 ng/ml, insufficiency as 25(OH) vit D between 20- 40 ng/ml and 25(OH) vit D sufficiency as 25(OH) D > 40 ng/mL. 25(OH) vit D deficiency (VDD + VDI) was observed in 93% of the subject population. Most of the subjects belonged to the age group of 41–60 years. 59% had frank 25(OH) vit D deficiency when cut off level was <20 ng/mL. Mean value of 25(OH) vit D in our subjects was 21.4 ± 14.4 ng/mL. Significant differences in 25(OH) vit D level were observed in between male and female subjects. Simultaneously 25(OH) vit D levels was significantly lower in the patient visited hospital in winter than visited in the summer session ($p > 0.001$). Conclusion: Our study demonstrates a high prevalence of 25(OH) vit D deficiency in an ostensibly healthy Indian population. There is a need for extensive improvement in status of vitamin D in our population.

S62

Cytoprotective Pathways in Diabetes Mellitus and its Vascular Complications: Transcription Factor Nrf2 & NAD(P)H: Quinone Oxidoreductase

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Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder strongly associated with macrovascular and microvascular complications. Hyperglycemia, acting through many metabolic and structural derangements leads to excessive production of reactive oxygen species (ROS), which is an early link between oxidative stress (OS), inflammation and diabetic complications. The capacity of a cell or an individual to manage OS is mediated through transcription factor Nrf2, which is a master switch leading to the coordinated expression of genes encoding antioxidant and phase 2 detoxifying enzymes such as NAP(P)H:Oxidoreductase-1 (NQO1) and Glutathione-S-Transferases(GSTs). NQOI is a FAD containing cytosolic protein which promotes obligatory reduction of quinones and thus preventing the depletion of intracellular GSH and GST. However, these endogenous antioxidant defense mechanisms are impaired in T2DM and its complications viz diabetic nephropathy. Cytoprotective aspects of these mechanisms and their differential expression in oxidative stress mediated disorders will be presented.

A1

Novel Diagnostic Model Using Iron Homeostatic Proteins for Differentiating Acute Bacterial Meningitis from Acute Viral Meningitis in Infants

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Meningitis is major health burden in infants in both developed and developing country. Differentiation between acute bacterial meningitis (ABM) and acute viral meningitis (AVM) has always remained a challenge citing its prognostic significance. Conventional parameters are usually time consuming and less specific. Most of the bacteria causing meningitis do modulate iron homeostatic proteins as a measure for its own survival. Exploiting changes made by bacteria in terms of iron and iron homeostatic proteins can provide a powerful diagnostic tool to differentiate between ABM and AVM. We tried to explore the diagnostic implications of evaluating iron, ferritin transferrin and ceruloplasmin and possibility of a model based on iron homeostatic proteins to differentiate between ABM and AVM. The objectives of the study were to evaluate the diagnostic role of iron and iron homeostatic proteins (ferritin, transferrin, and ceruloplasmin) in bacterial meningitis and to build a model based on iron homeostatic proteins to differentiate between ABM and AVM. The study included 32 infants each of ABM and AVM. Iron, Ferritin, Transferrin and ceruloplasmin were evaluated in both serum and cerebrospinal fluid(CSF) of the two groups. Patient's demographic data, serum and CSF values were represented as median and range. Iron and ceruloplasmin were measured by spectrophotometry. Ferritin and Transferrin were estimated by chemiluminescent immunoassay and ELISA respectively in both serum and CSF. We found CSF levels to be significantly elevated for iron, ferritin, and transferrin, in ABM than in AVM. On the other hand we found elevated transferrin as only significantly elevated parameter with respect to ABM than in AVM. Transferrin ratio (CSF transferrin/serum transferrin) was also significantly high in ABM. Ceruloplasmin ratio (CSF ceruloplasmin/serum ceruloplasmin) was low in ABM though nonsignificant. The AUC of Transferrin ratio was 0.774 with sensitivity of 66% and sensitivity of 84%. Ceruloplasmin ratio though non-significant had specificity of 97% and PPV of 92%. ROC Analysis of our best modelcomprising ferritin ratio, transferrin ratio and ceruloplasmin ratio showed area under curve (AUC) as 0.817, Sensitivity as 72%, Specificity as 85% and negative predictive value (PPV) of 82%. We hereby conclude that iron homeostatic proteins have diagnostic role in bacterial meningitis. The model obtained using iron homeostatic proteins was significant and comparable to existing models.

A2

Targeting Folate Metabolism: A Promising Therapeutic Rationale against *Brugia malayi* Infection

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Lymphatic filariasis although not fatal, is a profoundly disabling disease, with an estimated level of 5.8 million disability adjusted life year. Besides being almost sole drug, DEC have various limitations. Hence exploration of effective filarial target is now needful. Taking clue from earlier promising results with certain plant extracts having polyphenolic ingredients as well as synthetic compounds with potential DHFR inhibitory effect, we postulated a plausible link between folates and polyphenolics based on their common precursor in shikimate metabolism. With the perspective of structural similarity based antagonism, we have attempted to validate parasitic dihydrofolate reductase (DHFR) enzyme and associated folate metabolism for therapeutic target. For this purpose, initial bioinformatics approach for molecular docking and further validation by in vitro assay using green tea extract as a probe followed by folate reversal study and synergistic effect with piperidine compound. A comparative docking analysis between human and Brugia malayi DHFR showed remarkably effective binding parameters with lower inhibition constants of these ligands with parasitic target, but not with human counterpart suggesting safety and efficacy. The virtual results were further validated by significant in vitro antiparasitic effect of green tea extract followed by folate reversal assay and marked synergistic impact with piperidine compound confirming the involvement of folate pathway. Finally, we recorded the anti-folate induced apoptosis indicating impairment in DNA synthesis due to DHFR inhibition. Therefore, we conclude that DHFR might be considered as a valid target for drug design with reasonable safety and efficacy.

A3

Insulin Sensitivity Index ($ISI_{0,120}$) Potentially Linked to Carbon Isotopes of Breath CO_2 for Non-invasive Diagnosis of Pre-Diabetes and Type 2 Diabetes

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New strategies for an accurate and early detection of insulin resistance are important to delay or prevent the acute onset of type 2 diabetes (T2D). Currently, insulin sensitivity index ($ISI_{0,120}$) is considered to be a viable invasive method of whole-body insulin resistance for use in clinical settings in comparison with other invasive sensitivity indexes like homeostasis model assessment (HOMA), and quantitative insulin sensitivity check index (QUICKI). To investigate how these sensitivity indexes link the $^{13}C/^{12}C$-carbon isotopes of exhaled breath CO_2 to pre-diabetes (PD) and type 2 diabetes in response to glucose ingestion, we studied excretion dynamics of $^{13}C/^{12}C$-isotopic fractionations of breath CO_2. Here, we show that $^{13}C/^{12}C$-isotope ratios of breath CO_2 were well correlated with blood glucose, insulin, glycosylated-hemoglobin as well as with HOMA-IR and 1/QUICKI. Conversely, the strongest correlation was observed between $1/ISI_{0,120}$ and breath CO_2 isotopes. Consequently, we determined several optimal diagnostic cut-off points of $1/ISI_{0,120}$ and $^{13}CO_2/^{12}CO_2$-isotope ratios to distinctively track the evolution of pre-diabetes prior to the onset of type 2 diabetes. Our findings suggest that isotopic breath CO_2 is a novel method for accurate estimation of $ISI_{0,120}$ and thus may open new perspectives into the isotope-specific non-invasive evaluation of insulin resistance for large-scale real-time diabetes screening purposes.

O01

Prenatal Diagnosis of Genetic Disorders by Molecular Genetic Methods

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The burden of genetic disorders is high in India mainly due to the large population as well as practice of consanguineous marriages and founder effect of mutations in communities. Advances in molecular genetic technology help in identifying the genetic defect in the affected probands and lead to carrier screening and identification of high risk families who can benefit from the advances in genetics for prevention of genetic disorders by resorting to prenatal diagnosis.

In India most of the families with an affected child desire prenatal diagnosis. A pre-requisite for prenatal testing is identification of the underlying genetic etiology.

Once the Molecular genetic defect is known in a family, prenatal diagnosis can be done using the same technology for these for prevention of birth of a child with that disorder. Thus, prevention of the birth of affected children by prenatal diagnosis, to reduce the socio-economic pressure on the family and burden of the disease on the community is possible by genetic counseling and prenatal diagnosis (PND)

The various elements for a successful prenatal diagnosis program for genetic disorders in India will be discussed which include:

- Molecular studies in the affected child and the parents to identify the mutations responsible for the disease.
- Choosing the most suitable obstetric technique to obtain fetal tissue and performing the procedure.
- Extraction of DNA from the fetal tissue and carrying out molecular studies to establish the status of the fetus.
- Sources of error and their avoidance
- Verification of the result after birth or abortion.

These issues related to prenatal diagnosis of genetic disorders by molecular genetic methods will be discussed.

O02

Mining the Expression of Mycobacterial Region of Difference (RD) Proteins in Sputum of Pulmonary Tuberculosis (PTB) Patients

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The RD genes of mycobacteria are a group of proteins which are specifically present in Mycobacterium tuberculosis complex. Their specificity makes them attractive candidates for diagnosis. An insight into their expression *in vivo* in patient samples may highlight the relative importance of some specific RD proteins among the group of RD proteins and those proteins which are abundantly expressed in vivo may generate a good antibody response, hence serving as better serodiagnostic candidates.

The aim of present study was to identify highly expressed M. *tb* RD genes using Microarray in TB patient sputum that could be exploited as serodiagnostic markers of active TB.

Microarray was used for studying the gene expression. RNA was isolated from PTB patients and lung cancer patients. RNA was converted into labeled cDNA and used on the Microarray slide which was further scanned and analyzed using various softwares.

The gene expression profile of *M. tb* in smear positive sputum samples from TB patients showed 558 differentially-expressed genes as compared to *in vitro* grown H37Rv; 164 genes were upregulated and 394 genes were downregulated. As a negative control, microarray analysis was also performed on sputum samples from lung cancer patients In this analysis, 26 genes were detected as upregulated. Of these upregulated genes, 19 were also present in the TB sputum and therefore were excluded from our study. In the upregulated genes there were 6 RD genes, which can further be mined for their diagnostic potential.

Overall, 6 RD genes have shown increased expression in sputum of PTB patients and hence can be mined further for their serodiagnostic potential.

O03

Therapeutic Benefits of Nanotechnology Mediated Drug Delivery System Against Experimental Tuberculous Meningitis

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Tuberculous meningitis is the highest toll taking form of extra pulmonary tuberculosis. Though it represents roughly 1% of all cases of tuberculosis, it is disproportionately important because it kills or severely disables about half of the people affected. The conventional therapeutic regimen consists of Isoniazid, Rifampicin, and Pyrazinamide for a period of 6–24 months depending upon the complexity of the disease. Two major limitations to this therapy include limited ability of antitubercular drugs to cross the blood brain barrier and frequent dosing of drugs causing patient non-compliance. The current study was designed to develop drug delivery system which can enhance uptake of drugs across blood brain barrier. Oral and intravenous route were studied, to select the most suitable route for administration of the drug delivery system. Oral route was found to be equally efficient as the intravenous route. Drugs were administered orally in mice via drug delivery system. After single oral dose, sustained release of drug in the plasma was observed for up to 96 h and drug levels in the brain were detected for up to six days. Further murine model for tuberculous meningitis was developed to evaluate the therapeutic efficacy of the drug delivery system. Infected mice showed better recovery when treated with nano drug delivery system. Drug dosage frequency was reduced to $1/8^{th}$ and better clearance of infection was found in brain. *This is the first report demonstrating the implication of a novel nanoparticle based therapeutic formulation for the treatment of tuberculous meningitis.*

O04

Comparison of Urea Estimation by Two Different Methods (UREASE-GLDH-Fixed Time and Modified Berthelot) on Semi-automated Biochemistry Analyser

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1. To measure the serum urea levels by two methods (UREASE-GLDH-Fixed time and modified Berthelot)
2. To compare the results obtained by these two methods.
3. To compare the accuracy, precision and linearity of these two methods.

The study was conducted at Clinical Chemistry laboratory of S.S.G Hospital and Medical College, Baroda using QC sera, patients sample and urea estimation kits of GLDH and Berthelot methods on semi-automated biochemistry analyser. Human lyophilized QC sera was analysed 20 times by both the methods to find the precision. A serial sample of high urea level was taken and serial dilutions were done and analyzed to know the linearity of urea in both the methods. 40 samples of varying urea concentration were analyzed by both the methods and regression analysis was done to compare the accuracy of both the methods.

The mean ± SD (%CV) of 20 repeated tests of QC sera of Urease-GLDH-Fixed time method is 47.5 ± 2.06(4.35) and modified Berthelot is 49.5 ± 2.04 (4.11). Linearity of GLDH method is 10–350 and modified Berthelot is 8–340 mg/dl. Comparison of analytical accuracy between two methods gave a regression equation $y = 0.89x + 7.03$ with a correlation coefficient(r) of 0.88 and coefficient of determination (r^2) of 0.78.

Results of these two methods are comparable and both the methods are equally precise, have similar linearity and show high degree of correlation suggesting similar accuracy. Further studies are being done to know the various interfering factors in both these methods.

O05

Leptin Regulates Hypercholesterolemia in Alcoholic Liver Disease

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Alcohol induced fatty liver disease is the most common and earliest response to the progression of fibrosis, cirrhosis and/or hepatocellular carcinoma. The mechanism by which ethanol causes fatty liver disease is complex and not fully understood, however, enhanced hepatic lipogenesis has been proposed as an important biochemical mechanism. We have previously noted that the potential preventive effect of leptin on alcohol elicited toxicity in *in vitro*. The purpose of this study was to evaluate the effect of leptin on ethanol induced elevated hepatic cholesterol synthesis and fatty acid composition in mice. CD-1 mice (n = 15/group) were studied for 45 days. Four groups were studied. 1) control, 2) leptin + control (230 μg/kg intraperitoneal every alternate day from day 15), 3) alcohol (6.32 g/kg daily by gastric lavage, for 45 days) and 4) alcohol + leptin (as prior dosing). Compared to control, ethanol supplementation significantly ($p < 0.05$) increased % of palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1) and docosapentaenoic acid (22:5) levels, whereas palmitoleic acid (16:1) and arachidonic acid (20:4) concentrations were decreased significantly ($p < 0.05$). Leptin treatment to ethanol fed mice significantly corrects the above indices. Furthermore, leptin administration significantly down regulates ethanol induced plasma total and ester cholesterol and hepatic HMG CoA reductase, cholesterol ester synthase and sterol regulatory element binding protein 2 protein expression. The activities of hepatic lipoprotein lipase, plasma lecithin cholesterol acyl transferase and hepatic cholesterol ester hydrolase were significantly ($p < 0.05$) lowered following ethanol supplementation compared to control mice. These features were significantly ($p < 0.05$) increased by addition of leptin. Liver histology showed that mice given ethanol had macro and micro vesicular steatosis. However, ethanol + leptin treated liver showed sinusoidal dilatation and no fatty change. Conclusion: Thus, administration of exogenous leptin to alcohol fed mice significantly decreased hepatic cholesterol accumulation and also regulates fatty acid composition; warranting population based further mechanistic studies.

O06

Association of Single Nucleotide Polymorphisms of Two Enzymes (ACE I/D and renin MBbo I) of RAAS with Essential Hypertension

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Discovery of a pivotal role of renin and ACE (rate-limiting and key components of RAAS) in blood pressure homeostasis in experimental and clinical studies have suggested a prospective importance of molecular variants of these as a rational candidate for unravelling the genetic basis of essential hypertension. To evaluate the prototype and alliance of two single nucleotide polymorphisms (SNPs), namely an insertion/deletion polymorphism in intron 16 of the ACE gene and REN MboI polymorphism at the locus intron 9 in essential hypertension. Genotyping was performed by PCR-RFLP method for characterization of ACE I/D and REN MboI (10631A>G) gene polymorphism in a total of 67 hypertensive and 70 normotensive participants. No departure from Hardy-Weinberg equilibrium was observed in either cases or controls for both the studied SNPs. An increased frequency of mutant allele of ACE (p: 0091) and MboI of renin (p: 0.0239) was observed among patients affected with hypertension, which resulted in significant difference in genotypic ($p \leq 0.033$) and allelic ($p \leq 0.005$) distribution between cases and controls. A significant association was found for A/A variant (p: 0.0159) of MboI of renin gene and D/D variant (p: 0.0061) of ACE gene with essential hypertension and is substantiated by a statistically significant increase in odds of hypertension (renin: OR: 2.07; CI: 1.32–2.28; p: 0.03; ACE: OR: 2.19; CI: 1.47–2.51; p: 0.02) in multiple logistic regression analysis. Diallelic (I/D) polymorphism in ACE gene and genotype-associated differences in renin gene have functional consequences and thus can be used as a valuable genetic indicator for the susceptibility to essential hypertension.

O07

Role of *CYP1A1**2C (2455 A>G) Gene Polymorphism in Pathogenesis of Male Infertility

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Genetics is known to play a role in etiopathogenesis of male infertility. CytP450 is involved in metabolism of fertility hormones and many xenobiotics are known to have endocrine disrupting effect. Effect of a few polymorphisms of CytP450 has been evaluated and seen to have variable effect on male infertility.

The present study is designed to explore the effect of *CYP1A1**2C (2455 A>G) gene polymorphism on seminal parameters and thereby male infertility.

Male partner of infertile couple ($n = 80$) were evaluated for their sperm parameters as per seminal analysis method recommended by WHO (2010). *CYP1A1**2C (2455 A>G) gene polymorphism was assessed by ASO-PCR. Sperm parameters were compared by Kruskal Wallis test and risk of developing male infertility in polymorphic form of *CYP1A1**2C (2455 A>G) gene was calculated from odd's ratio.

Sperm count, motility and morphology were significantly affected in polymorphic genotype of *CYP1A1**2C (2455 A>G) gene when compared to wild genotype of *CYP1A1**2C (2455 A>G) gene. Odd's of developing male infertility among the polymorphic genotype was 3.96 (CI: 1.23–12.76; $P < 0.05$).

*CYP1A1**2C (2455 A>G) gene polymorphism alters the sperm characteristics and is a risk factor for male infertility.

O08

OLR1 Gene Polymorphism and Serum Levels of Oxidized ldl and Paraoxonase in Patients with Metabolic Syndrome

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Metabolic syndrome (MetS) is a cluster of the most dangerous cardiovascular disease risk factors: abdominal obesity, raised fasting plasma glucose, high serum Triglyceride, low HDL-C and high blood pressure. OLR1, a cell surface endocytosis receptor recognize, internalize and degrade oxidized LDL in vascular endothelium and plays a role in the pathogenesis of atherosclerosis. The antioxidant properties of HDLs are to some extent, attributable to serum Paraoxonase.

The aim was to explore the association of *OLR1* gene polymorphism in patients with MetS and also measure the serum levels of oxidized LDL and Paraoxonase in patients with MetS.

Forty cases fulfilling the IDF diagnostic criteria and 40 age and sex matched healthy controls were genotyped for *OLR1* gene (SNP: IVS4–73C>T, rs3736234) by RFLP-PCR. Serum oxidized LDL and Paraoxonase was estimated by ELISA. Association between the gene polymorphism and occurrence of MetS was estimated by Odds ratio, which was calculated by Unconditional Logistic Regression models.

The T allele of *OLR-1*: IVS4-73 C>T SNP is associated with significantly increased risk of developing MetS. (OR: 14.79, 95% CI: 1.80–121.2, $p < 0.05$). Serum oxidized LDL and Paraoxonase levels were significantly increased in the cases as compared to controls ($p < 0.0001$, 0.0003 respectively), but no association was found with the SNP.

The intronic SNP: IVS4-73 C>T of *OLR1* gene has significantly increased risk of developing MetS. Oxidized LDL and the antioxidant Paraoxonase might contribute in the pathogenesis of MetS.

O09

Twin Siblings with Succinic Semialdehyde Dehydrogenase (SSADH) Deficiency in an Indian Family

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Succinic semialdehyde dehydrogenase (SSADH) deficiency (OMIM 271980, 610045) is an exceedingly rare, autosomal recessive metabolic disorder of γ- aminobutyric acid (GABA) metabolism caused by mutations of aldehyde dehydrogenase gene (ALDH5A) on chromosome 6p22.3. Till date, only three cases of SSADH deficiency have been reported from Asia and none from India. We report SSADH deficiency in two old twin siblings.

Ten year old twin male twins, born to non-consanguineous Sikh couple with no significant family history presented with seizures and global developmental delay from early infancy. They were also noted to have behavioural problems and choreiform movements of limbs from second year of life. Seizures gradually decreased and spontaneously resolved by three year age. Their current gross motor developmental age is five years, fine motor developmental age is fourteen months, social developmental age is seven months and speech development is commensurate to thirteen months. Both children have microcephaly, poor eye-contact, hyperactivity and emotional lability. Their limbs are hypotonic. They have choreiform movements, predominantly of upper limbs with motor impersistence. Their Intelligence Quotients (IQ) are 43 and 38 respectively. MRI brain of the elder twin revealed T2/FLAIR hyperintensities of bilateral globus pallidi. Biochemical investigations revealed unremarkable hemogram, liver function, renal function and thyroid function tests. Acyl carnitine profiles of both children assesses by TMS were normal. Urinary GC-MS of both children revealed increased levels of 4-Hydroxybutyric acid, 3,4-Dihydroxybutyric acid,3-oxo 4-hydroxybutyric acid,4,5-dihydroxyhexanoic acid and glycine, which is clearly indicative of SSADH deficiency. Mutation study of ALDH5A1 gene revealed identical homozygous mutations on exon 3: c.608 C>T; p.P203L in both children. Both parents and elder female sibling had heterozygous mutations. The children are being managed supportively with speech and behaviour therapy and are on regular follow-up. Parents have been explained about the illness and genetic counselling has been done.

SSADH deficiency was first described by Jakobs, et al in 1981. In this condition, there is an excessive build-up of GABA and γ-hydroxybutyrate (GHB) which are incriminated in the causation of symptomatology. GABAtransaminase deficiency and homocarnosinosis. are two other closely related disorders of GABA metabolism which can be differentiated biochemically. Clinical spectrum of SSADH deficiency includes seizures, developmental delay, hypotonia, tremor, movement disorder, sleep disorders and neuropsychiatric manifestations. Our index children manifested global developmental delay seizures, movement disorder and behavioural problems. They

were atypical in that their seizures had become passive. Sleep disorders, though very commonly reported in SSADH deficiency, were absent in our children. The neuroimaging and GC-MS profile were classical for SSADH deficiency. ALDH5A gene mutation noted in our family has been reported earlier among one of the 35 mutations detected till date. Currently, patients with this entity are managed supportively. Prospective research therapies including vigabatrin, taurine, $GABA_B$ receptor antagonist SGS-742 and gene therapy.

Our report is unique in being the first report of SSADH from India with few distinct clinical atypicalities as noted above. The objective of our presentation is to highlight the fact that SSADH deficiency mimics a static neurological disorder. There is a crucial role of clinical suspicion, neuroradiology, GC-MS and genetic studies in management of this condition.

O10

Effect of APOE Polymorphism on Leptin in Alzheimer's Disease

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Leptin, a 16 kDa peptide hormone synthesised and secreted specifically from white adipose cells protects neurons against amyloid β induced toxicity, by increasing Apolipoprotein E (Apo E) dependent uptake of β amyloid into the cells, thereby, protect individuals from developing AD. The Apo E ε4 allele is a known genetic risk factor for AD by accelerating onset. It is estimated that the lifetime risk of developing AD increases to 29% for carriers with one ε4 allele and 9% for those with no ε4 allele. To determine the levels of serum leptin, cholesterol, low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) in the diagnosed cases of Alzheimer's disease (AD) and the association of them with cognitive decline and Apolipoprotein E (APO E) genotypes in AD. Serum levels of serum leptin, cholesterol, LDL-C and HDL-C along with APO E polymorphism were studied in 39 subjects with probable Alzheimer's disease and 42 cognitive normal individuals. AD group showed significantly lower levels of leptin ($p = 0.00$) as compared to control group. However, there was no significant difference in cholesterol, triglycerides, LDL-C, and HDL-C levels in AD and control groups. The frequency of ε4 allele in AD (38.5%) was found to be significantly higher than in control (10.3%). ε3 allele was more frequent than ε4 allele in AD and control group. ε3 allele was more frequent than ε4 allele in AD and control group.

O11

Polymorphism of ACE and CYP46A1 Genes in Risk Prediction of Cataract Among North Indian Population

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Cataract is a clouding that develops in the crystalline lens of the eye or in its envelope; varying in degree from slight to complete opacity and obstructing the passage of light. According to World Health Organization, cataract is the most common cause of blindness; accounting for 47.8% of the 161 million visually disabled people worldwide. The present study was carried out to investigate the association of ACE and CYP46A1 genes polymorphism with cataract cases and controls.

This study includes 103 cataract cases and 102 controls. ACE (rs 4646994) and CYP46A1 (rs 754203) genes polymorphism in cases and controls were evaluated by PCR-RFLP.

Frequencies of ACE ID, DD, II genotype in cataract cases and controls were 64.08%, 4.86%, 31.06% and 61.76%, 26.48%, 11.76% respectively. The CYP46A1 gene CT, CC, TT genotype frequencies were 48.54%, 8.73%, 42.72% in cases and 28.43%, 3.93%, 67.64% in healthy controls respectively. ACE DD, II genotype ($p < 0.001$, $p = 0.0008$) and CYP46A1 CT, TT genotype ($p = 0.003$, $p = 0.0003$) were significantly associated with cases and controls.

Findings of this study suggest that ACE and CYP46A1 genes polymorphism can be a predictive marker for early identification of population at risk of cataract. The potential role of ACE and CYP46A1 genes polymorphism as a marker of Susceptibility to cataract needs further studies in a larger number of patients.

O12

Genetic Evidence of Idiopathic Chronic Pancreatitis in Paediatric Patients

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Idiopathic chronic Pancreatitis (ICP) is a challenging and disappointing disease. It is characterized by recurrent episodes of acute pancreatitis often beginning in childhood. A family history of at least two other affected members, frequent presence of calcified stones in the pancreatic duct, and absence of known precipitating factors such as alcohol or gallstones characterizes Hereditary pancreatitis which follows an autosomal dominant pattern of inheritance with 80% penetrance. The precise prevalence of HP is still unknown.

In our study, appearance of 90 children cases in three years may be a sign of increased incidence of the disease or it may have resulted from improved clinical awareness. We screened 50 patients including 46 ICP and 4 HP patients for mutation in the exons of Cationic trypsinogen (PRSS1) and Serine protease inhibitor kazal type-1 (SPINK1) genes. N34S mutation in SPINK1 has already been shown to be present in both ICP and HP patients. We found 3 HP patients and 6 Idiopathic chronic pancreatitis patients having N34S mutation. A silent mutation (D162D) in exon 4 of PRSS1 gene was found in 3 ICP patients. Our results supports the concept of ICP as a genetic disorder, however, detailed studies for other candidate genes as well as yet to be identified genes using advanced technologies such as whole exome sequencing are required to understand the pathophysiology of the disease.

O13

Adipokines and Endothelial Progenitor Cells Interaction in Metabolic Syndrome?

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Adipokines and Endothelial Progenitor Cells (EPCs) are the new emerging markers for Metabolic Syndrome (MetS). Recent evidence suggests EPCs maintain endothelial function whereas adipokines are intermediaries that link insulin resistance to endothelial dysfunction. We studied EPCs based on surface antigens CD 34 and CD 133 (naive heamatopeotic lineage markers) and KDR (a mature stage, endothelial commitment marker).

To assess whether there exists an association between circulating EPCs and adipokines.

100 MetS cases (IDF criteria) and 50 healthy controls were selected for the study. Adipokines (HMW adiponectin. Chemerin, Omentin and Visfatin) were measured using ELISA. EPCs; CD 34+ KDR+, CD 34+ 133+, CD133+ KDR+ and CD 34+ KDR+ 133+ were enumerated using flow cytometry. Lipid profile, fasting blood sugar, HOMA-IR, hs-CRP and anthropometrics were measured.

HMW adiponectin was reduced whereas chemerin was elevated and correlated to key components of MetS. CD34+ KDR+ cells were decreased and emerged as the best predictor of CV risk and IR whereas CD34+ 133+ were elevated in MetS as compared to controls. CD34+ 133+ cells correlated positively with chemerin but negatively with HMW adiponectin and Omentin. Further CD34+ KDR+ cells were decreased with increase in chemerin. We did not find any correlation between visfatin and EPCs.

Study suggests, decrease in HMW adiponectin and increase in chemerin act as a trigger to induce the mobilisation of CD34+ 133+ cells from bone marrow, however their further maturation to CD34+ 309+ endothelial lineage cells in circulation may be affected due to the presence of CV risk factors resulting in exhaustion of these cells and endothelial dysfunction. This is the first report from India.

O14

Leptin and Adiponectin as Predictors of Metabolic Syndrome in Psoriasis: A Case-Control Study

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Psoriasis is a chronic, immune mediated inflammatory skin disease. It has been considered of late as a disorder with systemic inflammation that could contribute to various systemic events like Metabolic Syndrome (MetS). Adiponectin and leptin are cytokines that are mainly secreted from the adipose tissue. They are believed to be a link between obesity, insulin resistance and endothelial dysfunction. This study was undertaken to analyze the role of adiponectin and leptin in relation to MetS in psoriasis. We performed a case-control study on 60 psoriasis patients and 60 subjects without psoriasis (Control). Cases and controls were divided into four groups based on the presence and absence of MetS. Serum adiponectin and leptin levels were measured in all four groups. The overall serum adiponectin levels were significantly reduced and serum leptin levels were significantly increased in psoriasis patients when compared with controls (p value < 0.05). Serum leptin levels were found to be significantly different in the four groups (p value $= 0.05$). The highest mean value of serum leptin (32.05 ng/ml) was observed in psoriasis with MetS group and lowest mean value of leptin (18.74 ng/ml) in controls without MetS (p value $= 0.011$). The lowest mean value of serum adiponectin (8108.33 ng/ml) was observed in psoriasis with MetS group and highest mean value of adiponectin (10623.92 ng/ml) in controls without MetS. Hyperleptinemia and hypoadiponectinemia is associated with psoriasis. They may play a role in the pathogenesis of psoriasis and may also contribute to MetS in psoriasis.

O15

Association of Leptin and Adiponectin with Postpartum Depression at Six Weeks Post Delivery in a Nested Case-Control Study

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Postpartum depression (PPD) is a common disorder, with various biological etiology affecting 13–20% of women, which adversely affects the interpersonal relationship between women and her child. Leptin and adiponectin, the proteins synthesized by adipose tissue, with primary role in energy expenditure, have been related to depression in general population with conflicting results. The present study aim to evaluate the association of leptin and adiponectin with PPD at 6 weeks post delivery, with the goal of searching a potential biomarker for PPD.

This is a nested case-control study with 70 women as subjects (women with PPD (cases) $= 35$ and women without PPD(control) $= 35$). The diagnosis of PPD was done at 6 weeks post delivery using Edinburg Scale for Postnatal Depression (EPDS). Blood samples from these women were also collected at the same point. Serum leptin and adiponectin were performed using commercially available ELISA kits.

Leptin levels were found to be increased in women with PPD as compared to control, but was not statistically significant($p = 0.456$). There was no difference in the adiponectin levels between the two groups. Linear regression did not show any significant correlation.

Leptin may be a promising marker for PPD, but further larger cohorts are required to confirm the above findings.

O16

ART Induced Oxidative DNA Damage and Insulin Resistance

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Insulin resistance (IR) is frequent in HIV infection and may be related to Antiretroviral Therapy.

The present study is aimed to assess the prevalence of IR & its association with oxidative DNA damage & cART in a non-diabetic HIV-1 Patients.

The cross-sectional study was conducted in 200 treated HIV-1 infected, 100 untreated HIV-1 infected and 100 HIV-negative controls, aged 20–60 after obtaining their consent at the OPD and ART Centre of the GGMC, Mumbai. The protocol study was approved by the Institutional ethics committee and NACO Delhi, India. Sr. Insulin was determined using Immulite 1000 Insulin kit Siemens. IR was estimated by HOMA. The Oxidative DNA damage marker 8-OH-2-dG was determined by ELSA kit from StressMarq Biosciences. Data was analyzed using EpiInfo-7 software.

The normal range of plasma 8-OH-2-dG is 4–21 pg /ml. IR was estimated using the HOMA and a clinically significant cut-off 2.81 was used for HOMA-IR. Overall, the prevalence of DNA damage and Insulin resistance were significantly higher ($p > 0.001$) in HIV-1 positive patients undergoing ART than ART naive and controls.

We observed that ART induced oxidative DNA damage plays a significant role in the development of IR which leads to the consecutive risk of diabetes and cardiovascular events. Hence we suggest that the oxidative DNA damage & IR management should be a central component of HIV-infection therapeutic strategy.

O17

Identification and Molecular Characterization of Polymorphism in Type 2 Dopamine Receptor (DRD2), Serotonin Receptor (5HT2A) and CYP2D6 Gene Polymorphisms in North Indian Schizophrenic Population - A Multicentric Study

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Schizophrenia is a severe psychiatric disorder characterized by psychotic symptoms i.e. delusions, hallucinations, disturbances of thought, disorganized speech & behavior causing significant impairment in social and occupational functioning. Schizophrenia is associated with unknown etiology, complex pathophysiology, long lasting and poor treatment outcome. Worldwide prevalence of schizophrenia is about 1% in general population. The mode of inheritance of schizophrenia is complex as it is a multifactorial psychiatric disorder in which both genetic factors and environmental interactions are involved. Moreover there are few reports available on polymorphism studies on dopamine receptors, serotonin receptors and CYP2D6 genes in schizophrenia patients in India.

Genotypes of dopamine receptor type 2 i.e. Taq1A, Taq1B, Taq1D & S311C, serotonin receptors - T102C, C516T & A1438G and CYP2D6 gene polymorphisms- CYP2D6*3, CYP2D6*4 & CYP2D6*10 were studied by PCR based Restriction Fragment Length Polymorphism technique. In this study 443 schizophrenic patients, 443 unaffected first degree relatives of schizophrenic patients and 150 healthy normal individuals were enrolled for comparative analysis. The patients were evaluated on the basis of PANSS (Positive and Negative Syndrome Scale) by the Psychiatrist.

Genotype and allele frequencies were compared among schizophrenia patients, their first degree relatives and healthy random control samples in North Indian population by Chi-square ($\chi 2$) test. There is no significant association in any of the studied polymorphism with the disease except CYP2D6*4 gene polymorphism. The patients were categorized into responder, partial responder and non-responder groups based on reduction in baseline score of PANSS. None of the studied polymorphisms

O18

Electrochemical Impedance Based Genosensor for Early Detection of Bacteria Causing Damage of Human Heart Valves

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Rheumatic heart disease in initial stages manifests itself as sore throat. The disease is inflammatory in nature affecting many connective tissues, especially the heart valves. Usually the mitral or aortic heart valves are affected, becoming either stiff or leaky leading to obstruction of blood flow. It is caused by *Streptococcus pyogenes*, a Group A Streptococcus bacteria. Present diagnostic methods are biochemical tests (culture test, gram staining test), rapid antigen detection test, fluorescence in-situ hybridization and immunosensor. All these methods are time consuming, less sensitive and non specific as well as and non confirmatory based on single test. Therefore, impedimetric biosensors may prove as effective diagnostic technique of diagnosis.

To develop impedance based DNA sensors for detection of bacteria causing damage of human heart valves.

A specific and sensitive, impedance based sensor was fabricated for the early detection of *Streptococcus pyogenes* infection in human. The *mga* gene of *S. pyogenes* specific 24 mer ssDNA probe was covalently immobilized on dendrimer linked gold electrode through mercaptopropionic acid and electrochemical impedance was measurements at different concentrations of ssG-DNA. The sensor was characterized by FTIR and SEM.

The sensor exhibited a linear response to single stranded *S. pyogenes* DNA from 0.01 to 1 ng/6μL. The lower limit of detection of the sensor was 0.01 ng/6μL.

The genosensor can save damage of mitral and aortic heart valves of patient by early diagnosis and treatment.

O19

Novel Gaseotransmitter's (H_2S) Specific Action on Mitochondrial Subpopulation Against Myocardial Ischemia Reperfusion Injury

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Hydrogen sulfide (H_2S), a novel third endogenous gaseotransmitter, proven to be cardioprotective against ischemia reperfusion (I/R) induced injury and apoptosis, primarily by preserving the mitochondria. Recently researchers were observed the

presence of two populations of mitochondria (i.e. interfibrillar (IFM) and subsarcolemmal (SSM)) in the heart, which behaves distinctly during physiology and pathology. Thus the present study is designed to understand the specific action of H_2S conditioning on these subpopulation in exerting its cardioprotection.

Male Wistar rats were subjected to I/R injury with or without H_2S treatment during pre-/post-ischemic period and analysed the outcome measures such as haemodynamic, infarct size, cardiac injury markers release, mitochondria physiology, ETC enzymes activity and mitochondrial respiration. Apoptosis was determined using DNA fragmentation assay.

H_2S pre- and post- ischemic treatment shows cardioprotection confirmed by improved LVEDP, reduced infarct size, creatine kinase and lactate dehydrogenase activities. Among the subpopulation, IFM shows higher complex I and complex III activities. H_2S pre-conditioning attenuates I/R induced decline of complex I and complex IV activities and rise of complex II activity in IFM but not in SSM. Diminished complex I, III and IV activities in I/R rat heart were recovered with H_2S POC in both SSM and IFM. In addition, H_2S pre- and post-ischemic treatments, irrespective of the energy status, the mitochondrial membrane potential was near to the normal level. SSM from rat hearts exposed to H_2S_IPC showed higher swelling than IFM in non-energized and succinate energized conditions, unlike H_2SPOC, where both shows substantial reduced swelling.

H_2S pre-conditioning exerts its cardioprotective effect by preserving the IFM subpopulation, however H_2S post- conditioning could recover both IFM and SSM.

O20

Magnesium Status in Hospitalized Patients with Special Reference to Waterborne Magnesium

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Various studies have reported low serum magnesium and myocardial magnesium and abnormal magnesium tolerance tests in patients with acute myocardial infarction. Magnesium therapy has been advocated but not yet confirmed. There are reports regarding low levels of serum magnesium in hospitalized patients. The present study focuses on the status of serum magnesium in ICU and ICCU admitted patients and its correlation with water borne magnesium.

To assess the magnesium status in ICCU patients and its association with water borne magnesium, present study was carried out in clinical biochemistry lab, SMIMER. All relevant information was collected. Blood samples were analyzed for renal, cardiac profiles and magnesium and water magnesium levels were analyzed. The results are expressed as Mean and SD.

There were 185 healthy subjects, 93 ICCU and 279 non-ICCU hospitalized patients. We observed significant hypomagnesaemia among filtered water users in healthy subjects (1.77 ± 0.36) $p < 0.01$, in ICCU patents (1.35 ± 0.44) $p < 0.01$, in non-ICCU hospitalized patients (1.51 ± 0.59) $p < 0.01$. We didn't observed significant difference in other parameters in filtered water users among these groups.

Significant hypomagnesaemia was observed among filtered water users in all the subjects (ICCU, non-ICCU and healthy subjects) with 42% prevalence of hypomagnesaemia in ICCU patients.

O21

Status of Vitamin D Related to Demographic, Dietary and Life Style in the Garhwal Region of Uttrakhand

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Status of Vitamin D is of interest when studying the epidemiology of illness in population groups because Vitamin D is currently documented to decrease the risk of diseases such as osteoporosis, cancer, and cardiovascular disease.

The objective of this study was to evaluate the associations between serum Vitamin D status (deficiency and insufficiency) and distinct demographic, dietary, and lifestyle characteristics of adults in the Garhwal region of Uttrakhand.

The study sample consisted of 200 adults aged 20–60 who had serum 25(OH) D measured and who had completed various questionnaires concerning dietary intake of Vitamin D and other lifestyle factors. Multivariate logistic regression was used to estimate the odds ratio (OR) of Vitamin D deficiency, insufficiency, and sufficiency in adults based on distinct demographic, dietary, and lifestyle characteristics. Statistical significance was set at $\alpha < 0.05$.

The occurrence of Vitamin D deficiency was higher in obese adults than in underweight to normal weight adults ($40.22\% \pm 5.07$ vs. $29.3\% \pm 3.91$), higher in adults who reported no sunburns than in adults who reported ≥ 3 sunburns ($43.39\% \pm 4.02$ vs. $18.0\% \pm 2.92$), and higher in adults who use sun protective measures regularly than in adults who do not ($50.1\% \pm 4.03$ vs. $28.0\% \pm 2.68$). The prevalence of Vitamin D deficiency increased as dietary intake of Vitamin D decreased.

Significant positive associations were found between Vitamin D deficiency and several characteristics, namely obesity (OR = 7.43, 95% CI = 4.33–12.77), physical inactivity (OR = 1.63, 95% CI = 1.03–2.58) poor dietary Vitamin D intake (OR = 2.34, 95% CI = 1.44–3.81), non-supplement use or supplement use with a low amount of Vitamin D (OR = 1.75, 95% CI = 1.05–2.89), and activities that decrease exposure to sunlight (from OR = 2.97, 95% CI = 2.14–4.13 to OR = 5.30, 95% CI = 3.17–8.85).

The results of this study demonstrate that obesity, physical inactivity, poor dietary intake of Vitamin D, and low sunlight exposure increases the risk for Vitamin D deficiency.

O22

Efficacy of Vitamin E on Nitric Oxide and Oxidative Stress Markers in North Indian Elderly

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Reactive oxygen species mediated cellular and biomolecular deterioration are now accepted to involve in aging process. It is conceivable that vitamin E supplementation reduces the age related modifications by reducing oxidative stress. However, the outcomes of clinical trials with vitamin E in age related disease prevention have been mixed. The present study was undertaken to assess the markers of oxidative stress i.e. serum paraoxonase, erythrocyte malondialdehyde, plasma uric acid, vitamin C, E and nitric oxide levels in the blood samples of different age group subjects and to investigate the

effect of vitamin E supplementation in ameliorating the levels of these markers in middle aged and elderly subjects. These parameters were estimated by using standard methods in 30 healthy younger individuals (20–30 years) served as controls and in 60 healthy subjects categorized into two groups i.e. Group I (40–55 years) and Group II (≥56 years) before and after 3 months of vitamin E supplementation. The obtained values were compared statistically by using student's t-test. Vitamin E supplementation (200 mg/day) brought about an improved antioxidant status with significantly raised plasma vitamin C, E, nitric oxide and serum paraoxonase levels ($p < 0.05$), and simultaneously depleted levels of plasma uric acid and erythrocyte malondialdehyde ($p < 0.05$) in middle aged and elderly subjects. These findings support the protective and anti-aging role of vitamin E supplementation in reducing oxidative stress in the study group subjects.

O23

Effects of Lead on Biochemical Parameters in Battery Manufacturing Workers of Western Maharashtra, India

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Lead inhibits enzymes of haem biosynthesis, alters haematological parameters, induces oxidative stress and alters the antioxidant status of battery manufacturing workers (BMW).

The main aim of this study is to know the present status of blood lead (PbB) levels and its effect on haem biosynthesis related parameters such as erythrocytes δ-aminolevulinic acid dehydratase (δ-ALAD), urinary δ-aminolevulinic acid (U-δALA) and porphobilinogen (PBG), haematological parameters, oxidative stress parameter i.e serum lipid peroxide and antioxidant parameters such as, RBC- superoxide dismutase, RBC- catalase, plasma ceruloplasmin and serum nitrite of BMW.

Forty BMW from Western Maharashtra, India, having age group between 19 and 42 years were selected as study group and compared with age matched 38 healthy male subjects (control group). From both group subjects, 10 ml blood sample was drawn by puncturing the anteriorcubital vein and the PbB, erythrocytes δ-ALAD, urinary δ-ALA and PBG and haematological parameters, Serum Lipid Peroxide (LP), RBC-Superoxide Dismutase (SOD), RBC-Catalase (CAT), Plasma Ceruloplasmin and Serum Nitrite were measured by using standard methods. Statistical analysis: Between controls and BMW group was carried out by students 't' test.

Blood lead levels of BMW showed significant elevation ($p < 0.001$, 1050%) as compared to controls. Activated δ-ALAD ($p < 0.001$, −58.88 %), non-activated δ-ALAD ($p < 0.001$, −62.06 %) showed significant decrease and ratio of activated to non-activated δ-ALAD ($p < 0.05$, 29.26 %) revealed significant increase in BMW as compared to controls. Urinary δ- ALA ($p < 0.001$, 161%) and Urinary-PBG ($p < 0.05$, 45.3%) concentrations showed significant increase in the study group as compared to the control group. In battery manufacturing workers, Hb ($p < 0.001$, −16.67%) PCV ($p < 0.001$, −20.31%) MCV ($p < 0.05$, −4.27%) MCH ($p < 0.05$, −5.66), MCHC ($p < 0.001$, −7.16%) and RBC count ($p < 0.001$, −10.39 %) revealed significant decrease, while a significant elevation was seen in the total WBC count ($p < 0.001$, 20.47%) as compared to the controls. Serum LP levels were significantly increased ($p < 0.001$, 96.86%) and all antioxidant status parameters such as RBC-SOD ($P < 0.001$, −26.32 %), RBC- Catalase ($P < 0.001$, −51.57%), Plasma Ceruloplasmin ($P < 0.001$, −35.13%) were significantly decreased and serum nitrite levels ($p < 0.001$, 154%) were significantly increased in battery manufacturing workers as compared to control subjects.

Blood lead levels continue to remain high in BMW, in spite of modern techniques used to reduce the lead exposure which inhibits haem biosynthesis, alters haematological parameters, and it induced oxidative stress and altered antioxidant status of battery manufacturing workers.

O24

IGF-1 Polymorphism and Circulatory IGF-1 Levels Increases Acne Severity

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Acne is the most common diagnosis made by dermatologists. Insulin like growth factor- 1 (IGF-1) plays an important role in regulation of androgen activity. It potentiates peripheral androgen signaling by induction of 5α-reductase activity and by activation of androgen receptors. IGF-1 activates phosphoinositide 3-kinase (PI3 K) and MAPK/ERK pathways and thus mediates the increase in sterol response element binding protein- 1 (SREBP-1) mRNA, protein and lipogenesis. The incidence and severity of acne strongly correlates with the levels of circulating IGF-1. A polymorphism of the IGF-1 gene consisting of a highly polymorphic microsatellite composed of variable cytosine adenosine (CA) repeats situated in the promoter region 1-kb upstream from the transcription site of IGF-1 may directly influence the expression of IGF-1. We investigated the prevalence of IGF-1 polymorphism in acne patients and its influence on IGF-1 levels, and acne severity.

Eighty acne vulgaris patients, 20 cases each in the mild, moderate, severe and very severe grade as per Global acne grading score (GAGS) and 80 age and gender matched non-acne controls without any disorder or any history of drug intake likely to affect IGF-1 level were included in the study. The plasma levels of IGF-1 were estimated by ELISA and polymorphism was assessed by analysis of the size of the amplified product by polymerase chain reaction by PAGE.

Mean plasma IGF-1 level was significantly higher ($p = 0.041$*) in acne patients as compared to acne controls and it positively correlated with severity of acne ($p = 0.015$*). Homozygous carrier of 192-bp allele had 3.57 times Odds risk (95% CI; 1.37 - 9.32) of developing acne and had a higher mean level of IGF-1 compared to non-carrier of 192-bp allele. Homozygous or heterozygous carriers of 194-bp were not seen to be significantly susceptible to have acne. Both 192-bp allele and 194-bp allele carrier status did not show any significant association with the severity of acne.

Plasma IGF-1 levels correlate with severity of acne. Homozygous carriers of 192-bp allele had higher risk of developing acne. However IGF-1 polymorphism does not determine acne severity. Functional studies showing the relationship between IGF-1 promoter level polymorphism and actual gene expression in skin is warranted.

O25

Implications of Biomarkers Osteoprotegerin and Receptor Activation of Nuclear factor Kappa B Ligand in the Pathogenesis of Osteoporosis

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Estrogen deficiency is a major contributory factor to post-menopausal osteoporosis. It stimulates osteoprotegerin (OPG) secretion from osteoblasts and inhibits the production of nuclear receptor ligand kappa B (RANKL) production. OPG, RANKL are the final effector proteins of osteoclastic bone resorption and play a critical role in the regulation of bone turnover. The importance of these two biomarkers in the pathogenesis of postmenopausal osteoporosis is controversial. We studied the associations of serum OPG, RANKL and their ratio with bone mineral density (BMD) in healthy women. To examine the association between circulating levels of osteoprotegerin (OPG) and sRANKL and their ratio with ageing, bone turnover and bone density in elderly women. The study was performed on a group of 127 women aged 25–65 years. Baseline evaluation of bone mineral density (BMD), OPG, sRANKL, estradiol, and the bone markers osteocalcin (OC) and C-terminal telopeptide (CTX) were estimated. Serum OPG levels increased with age ($p < 0.001$) and were high in postmenopausal women associated with estrogen deficiency during menopause. sRANKL did not show a significant difference in various age groups. An increase in the OPG/RANKL ratio was observed ($P < 0.01$) in elderly women (age 40–65 years). sRANKL levels peaked during 46–50 years and dropped reflecting increased rate of bone loss. A negative correlation between the OPG levels BMD and T score and estradiol, a positive correlation between OPG levels and bone marker (OC, CTX) in postmenopausal women was observed. OPG levels increased with age and were high in postmenopausal women indicating an increased rate of bone loss. OPG and sRANKL levels were inversely related to BMD and positively with bone markers. The OPG: RANKL ratio can aid in identifying women at risk for osteoporosis and levels may play a role in increasing bone turnover in postmenopausal women.

O26

VEGF, PIGF and sFlt-1–A Diagnostic Marker for Pregnancy Induced Hypertension

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Preeclampsia is the major cause of maternal and fetal/neonatal mortality and morbidity even in developed countries. Despite extensive research, the etiology and pathogenesis of preeclampsia are not completely understood. Evidence shows that an imbalance between circulating angiogenic and anti-angiogenic factors, plays a central role in the pathogenesis of the disease. Since untreated preeclampsia may get complicated to eclampsia, a regular analysis using a marker that can predict real state and severity is essential. So a study has been designed to determine the circulating levels of Vascular endothelial Growth factor (VEGF), Placental growth factors (PIGF) and soluble form of VEGF receptors (sFlt-1) in preclamptic and eclamptic condition and to analyze whether it can be used a marker to predict the severity.

Study group consisted of Normotensive pregnant women (N) preclamptic women (PE) and Eclampticwomen (E) with 100 subjects in each group. Serum of VEGF, PIGF and sFlt-1 were determined by ELISA method using commercially available kit. Statistical analysis was made by one way analysis of variance.

When compared to control, sFlt-1 level was found to be significantly high and VEGF&PIGF level was found to be significantly low in PE and E group. Eclamptic pregnant women were found to have a significantly high Plasma level of sFlt-1 and low level of VEGF level when compared to preeclamptic group.

Our study substantiated the fact that imbalance in the pro angiogenic and anti angiogenic factor exist in PIH condition and identified that VEGF, PlDF and sFlt-1 can be used as a marker to analyze the severity.

O27

OxLDL Activates Apoptosis Signal Regulating Kinase1 (ASK1) via PLCβ2 and Calcium Signaling in Human Platelets

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OxLDL activates blood platelets but the underlying signalling mechanisms remain obscure. Apoptosis Signal Regulating kinase1 (ASK1) is a cytosolic MAPKKK present in human platelets which gets activated by phosphorylation at threonine $_{838}$ residue under condition of oxidative stress. OxLDL is also generated by free radicals *in vivo* and has implications in atherosclerosis.

The objective of the study was to investigate if OxLDL activates ASK1 in human platelets and its underlying signalling mechanism.

Whole blood was drawn into ACD from healthy volunteers under informed consent. Washed human platelets were obtained in tyrodes buffer (pH 7.3) by standard protocol. LDL was isolated from plasma by precipitation method using heparin citrate buffer pH 5.0 and oxidized with Cu at 37°C for 24hrs. Platelet aggregation studies were done using chronology lumiaggregometer. For signalling studies, immunoblotting was done of the platelet samples treated with different molecules i.e. OxLDL, n-LDL, PLCβ2 inhibitor, MEK1 inhibitor, BAPTA-AM, thapsigargin and CD36 function blocking antibody. Blots were incubated with different primary antibodies and detected with ECL. Results were obtained with densitometry analysis of at least 4 independent experiments ($p < 0.05$).

OxLDL robustly activated ASK1 in human platelets. The downstream MAPKs i.e. JNK1/JNK2, p38 and ERK2 were also phosphorylated and activated by OxLDL. ASK1 activation was significantly inhibited by PLCβ2 inhibitor and quenching of cytosolic calcium by BAPTA-AM. Similar inhibition was observed in case of JNKs and p38. CD36 function blocking antibody didn't inhibit ASK1 activation suggesting that signal might not relays through CD36 receptor on platelets. OxLDL only in combination with 20uM ADP showed significant platelet aggregation.

OxLDL is a robust agonist for ASK1 activation and lies downstream of PLCβ2 and calcium signaling in human platelets, suggesting that ASK1 is a calcium sensitive kinase.

O28

Assessment of Beta-Cell Function in Thalassemic Children

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β thalassemia major is widespread throughout the Mediterranean region, Africa, Middle East, Indian subcontinent, and South East Asia. In thalassemics, iron overload of tissues due to frequent blood transfusions leads to progressive dysfunction of the heart, liver, and endocrine glands. Disturbances of glucose metabolism are frequent in these patients. However, there is no consensus regarding etiology of abnormal glucose metabolism. Though insulin deficiency has been proposed as the causative factor, some studies report presence of insulin resistance and hyperinsulinemia.

The present study was conducted to assess the pancreatic β-cell function in thalassemic children.

The present study was conducted in 60 chronically transfused non-diabetic thalassemic children attending thalassemia clinic, Rajindra Hospital, Patiala. 30 age and sex matched healthy children were taken as controls. Fasting plasma glucose, fasting plasma insulin and serum ferritin levels were assessed in all the children. β-cell function index was calculated using the HOMA 2 calculator based on homeostasis model assessment (HOMA).

Plasma insulin levels were significantly higher in the study group as compared to the control group ($p = 0.0003$). β-cell function index was higher in the study group as compared to the control group, but this difference was not statistically significant ($p = 0.1352$). β-cell function index correlated well with serum ferritin and plasma insulin levels ($p < 0.0001$, highly significant).

It might be suggested that insulin resistance precedes the glycemic abnormalities in thalassemic children. This state of insulin resistance may overwork the beta cell function and in addition to iron toxicity, leads to impaired glucose tolerance and diabetes mellitus later.

O29

CVD Risk Beyond LDLc: Non-HDLc, Insulin, Insulin Resistance (IR) and Apo-Proteins in Overt Hypothyroidism

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The aim of present study was to analyse the cardiovascular risk markers beyond LDLc like non-HDLc, insulin, IR and apo-proteins.

Several known limitations make LDLc a less accurate marker of cardiovascular risk than either non-high-density lipoprotein cholesterol (non-HDL-C), or apolipoprotein B (apoB). There are no reported studies of these markers in overt hypothyroidism from India.

The study included 150 overt hypothyroid patients diagnosed for the first time and 100 healthy controls. The hypothyroid patients were recruited from the medicine outdoor based on their thyroid profile and clinical symptoms. All study subjects were analysed anthropometrically and clinically for weight, height and blood pressure. The fasting blood samples were analysed for blood sugar, insulin, lipid profile, C-peptide, apo-B and apo-A_1. The study results were statistically analysed using mean, SD and Pearson's correlation analysis.

We observed the hypothyroid patients to have significantly raised BMI ($p < 0.0001$), hypertension (SBP $p < 0.0001$) (DBP $p < 0.0001$) as compared to healthy controls (HC). There was gross dyslipidemia in hypothyroid patients as compared to HC ($p < 0.0001$). Hyperinsulinemia and IR was observed in hypothyroid patients as compared with HC ($p < 0.0001$). Correlation analysis showed a positive significant association of non-HDLc with other potential CVD risk markers like SBP ($r = 0.279$, $p = 0.0005$), DBP ($r = 0.289$, $p = 0.0005$), insulin ($r = 0.652$, $p < 0.0001$), HOMA-IR ($r = 0.652$, $p < 0.0001$), apo B ($r = 0.528$, $p < 0.0001$) but non-significant association with BMI ($r = 0.161$, $p = 0.49$) and apo-A_1 ($r = 0.124$, $p = 0.13$).

Non-HDLc and apo-proteins can prove to be significant tools in a physician's armoury while evaluating a newly diagnosed hypothyroid patient's CVD risk, since LDLc may at times mislead.

O30

Additional Supplementation of Water Soluble Vitamins in Hypothyroidism: Effects on Biochemical Profile and Clinical Outcome

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Hypothyroidism is associated with oxidative stress and endothelial dysfunction. Treatment with levothyroxine increases metabolic turnover and can hence transiently worsen these conditions.

This study was undertaken to analyse the effect of additional supplementation with water soluble vitamins on biochemical profile and clinical outcome in the initial phase of treatment of drug naïve patients with overt hypothyroidism.

This was a single blinded randomized controlled study. 56 newly-diagnosed drug-naïve overt hypothyroid women between the age of 25 and 45 years attending the Endocrinology OPD of JIPMER, Puducherry were randomized into two groups receiving water-soluble vitamins and placebo respectively, in addition to standard therapy (Levothyroxine 100 μg/day). Blood sample was obtained at baseline and after one month and two months of treatment. 28 age and BMI matched euthyroid controls were also recruited and a single baseline blood sample was obtained from them.

Overt hypothyroidism was found to be associated with oxidative stress, dyslipidemia, endothelial dysfunction, insulin resistance, and low-grade inflammation. A surge in oxidative stress and endothelial dysfunction was found in the initial phase of treatment with levothyroxine. Additional supplementation with water-soluble vitamins successfully ameliorated this surge. There was no difference between patient observed side effects between vitamin-supplemented and placebo-supplemented groups.

Additional supplementation of high doses of water-soluble vitamins to levothyroxine therapy may be of benefit in preventing

increase in endothelial dysfunction and oxidative stress in the initial phase of treatment of drug-naïve overt hypothyroidism.

O31

Osteoblastic Differentiation Requires Estrogen Receptor Alpha (ER-α66) and is Accelerated by Estrogen and Resveratrol

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Bone remodeling, a multistep process is regulated by many factors and estrogen is known to have a critical role. Deficiency of estrogen in postmenopausal women leads to the development of osteoporosis. The initial event in bone remodeling is the differentiation of precursor osteoblasts which is known to be regulated by RunX2 a 'master regulator'. While it is known that RunX2 and ER-α66 interact, the role of ER-α66 in the differentiation of precursor osteoblasts is poorly understood. Here, we highlight the importance of ER-α66 using cell lines and two of its ligands estradiol and resveratrol and by assessing its levels in the PBMCs of postmenopausal women.

To elucidate the role of ER-α66 in the differentiation of precursor osteoblasts in response to estrogen and resveratrol using two fetal osteoblastic cell lines of which one is deficient in ER-α66 isoform. To understand its role in postmenopausal osteoporosis we evaluated the expression level of ER-α66 and some of the candidate genes in the peripheral mono nuclear cells (PBMCs) of osteoporotic as well as non-osteoporotic postmenopausal women.

The fetal osteoblastic cell lines hFOB/ER9 (expressing ER-α66) and hFOB1.19 (deficient in ER-α66) were grown in DMEM upto about 80% confluence after which the cells were treated in the presence of either 1 μM resveratrol or 10 nM estradiol for 48 hours for use in the following assays. Untreated cells were used as control. The osteogenic differentiation was evaluated by alizarin staining to assess the mineralization process in both the cell lines. The osteoblastic activity was assessed by determining the activity of alkaline phosphatase (ALP). The promoter occupancy of the candidate osteogenic genes by ER-α was assessed by chromatin immunoprecipitation (ChIP) assay using ER-α antibody. The ChIP assay results were validated by analysing the expression of the candidate genes by quantitative Real-Time PCR. Further we also evaluated the expression level of ER-α and some of the candidate genes in the PBMCs isolated from EDTA treated blood samples of osteoporotic and non-osteoporotic postmenopausal women.

The estradiol and resveratrol either alone or in combination have potential to accelerate the differentiation of only hFOB/ER9 cells. Although there is over expression of RunX2 in hFOB1.19 cell line which lacks ER-α66, when present alone RunX2 is inefficient to accelerate osteoblastic differentiation as shown by the alizarin staining, ALP activity and ChIP assays which were validated by gene expression analysis. The gene expression pattern of ER-α 66 and other genes is different in the PBMCs of osteoporotic postmenopausal women.

ER-α66 has the potential to activate the osteoblastic differentiation in ligand independent manner but when bound to its ligands (estrogen or resveratrol) the process is further accelerated. RunX2, a master regulator of osteoblastic differentiation, is effective only when ER-α66 co-exists. Altered ER-α66 levels could be responsible for postmenopausal osteoporosis.

O32

NISCH and *CDH1* Promoter Hypermethylation in Lung Cancer: A Case Control Study

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The high mortality of lung cancer is attributable to presence of metastatic disease in nearly two-thirds of patients at diagnosis. Detection of early stage lung cancer amenable to curative resection could boost survival. Cancer specific methylation patterns of tumor suppressor genes, which precede precursor lesions, could possibly herald earlier diagnosis.

Present study was carried out to evaluate the frequency of promoter hypermethylation of *NISCH* and *CDH1* in cfDNA of lung cancer patients and correlate with clinicopathological variables.

Forty histopathologically confirmed lung cancer cases, thirty smoker and thirty non-smoker controls were enrolled. Plasma cfDNA was extracted and subjected to bisulfite treatment followed by MS-PCR. Statistical analysis was performed using SPSS22.0.

The promoter hypermethylation of both *NISCH* and *CDH1* was significantly higher in lung cancer patients ($P < 0.05$). *NISCH* was methylated more frequently in non-cancerous smokers as compared to lifelong nonsmoker controls ($P < 0.05$) but was independent of the smoking status of cancer cases. Pack years and packs per day were significantly higher in the methylated group. No significant association was found type or duration of smoking or with staging or histological grading.

NISCH and *CDH1* are highly methylated in plasma cfDNA of lung cancer patients and hence could be used as a part of blood-based biomarker panel for early diagnosis. Since *NISCH* is highly methylated in both high risk smoker controls as well as cancerous-nonsmokers, *NISCH* methylation may mark the convergence of varied etiologies of lung cancer. It may be investigated as a universal therapeutic target for lung cancers regardless of clinicopathological heterogeneity.

O33

Molecular Sub-types of Breast Cancer in South Indian Population

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Molecular phenotype of breast cancer is done on the basis of estrogen receptor (ER), progesterone receptor (PR), and HER-2. ER positive and/or PR positive and HER2 negative are classified as luminal A, ER positive and/or PR positive and HER2 positive are luminal B cancers, ER and PR negative and HER2 positive are considered as HER2 type. Cancers which are negative for ER, PR and HER2 are known as triple negative breast cancers (TNBC).

This study was carried out to identify the molecular sub-types of breast cancer in South Indian population and to compare the survival pattern of different molecular sub-types of breast cancer.

Three hundred and sixty eight (368) sporadic breast cancer patients were enrolled into the study. ER, PR and HER-2 were done

by immunohistochemistry. All relevant clinical and pathological data on the patients were collected.

The proportion of different categories were as follows – Luminal A – 125 (34%), Luminal B– 81 (22%), HER-2 type –79 (21.5%), and TNBC –83 (22.5%). Follow-up information were available for 179 patients which included luminal A – 66 patients, Luminal B – 44, HER-2 type 40 and TNBC 29 patients. Overall survival rate was 126 out of the total 179 patients (70%). The survival rates were as follows – Luminal A type – 55/66 (83%), luminal B type – 25/44 (57%), HER-2 type 20/40 (50%) and TNBC – 26/29 (90%).

Identification of molecular sub-types helps in planning treatment and predicting prognosis. TNBC and luminal A sub-types had better overall survival compared to luminal B and HER-2 sub-types.

O34

Upregulated Expression of rno-miR-96/182/183 Cluster in Liver of N-nitrosodiethylamine Treated Wistar Rats Plays a Role in Progression Rather than Initiation of HCC

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Hepatocellular Carcinoma (HCC) is a primary liver cancer and third leading cause of cancer related mortality worldwide. Incidence of HCC is increasing globally as well as in India. Molecular pathogenesis of HCC is still not completely understood. It has been shown that miRNAs plays a vital role in HCC. Among various HCC associated miRNAs, upregulated miR-96/182/183 cluster plays a role in hepatocarcinogenesis by targeting various genes like RASA1, CEBPα (hsa-miR-182), PDCD4 (hsa-mir-183), EphrinA5 (hsa-miR-182 & 96) etc. but the temporal analysis of miR-96/182/183 cluster expression has not been done previously.

To do temporal analysis of rno-miR-96/182/183 cluster expression during progression of liver disease from fibrosis, cirrhosis, dysplastic nodule followed by HCC in Diethylnitrosamine(DEN) treated Wistar rats to determine whether rno-miR-96/182/183 cluster plays a role in initiation or progression of HCC or both.

Study included 88 Wistar rats including 28 control and 53 DEN treated rats (via. intra-peritoneal injections at 50 mg/kg body weight weekly). Two rats from control and treated groups were sacrificed at 2^{nd}, 4^{th}, 6^{th} and 8^{th} weeks. Three control rats and five treated rats were sacrificed per week starting from 10^{th} week till 18^{th} week. Quantitative Real time PCR was done to determine expression of rno-miR-96/182/183 cluster in rat liver and data was normalized to RNU6B expression. H&E staining was performed to determine liver histopathology.

This study found non-significant deregulation of rno-miRNA-96/182/183 cluster expression from 2^{nd} till 15^{th} week (pre HCC stage) but significant upregulation was found from 16^{th} till 18^{th} week (HCC stage) i.e 16^{th} wk (p-value <0.001 for rno-miR-96/182/183), 17^{th} wk (p-value <0.05 for miR-96 and <0.001 for miR-182), and 18^{th} wk (p-value <0.05 for miR-182; <0.001 for miR-96 and 183) in liver of DEN treated rats.

Our study indicates that rno-miRNA-96/182/183 cluster being significantly upregulated from 16^{th} till 18^{th} week (HCC stage) in liver of DEN treated rats plays a role in progression rather than initiation of HCC.

O35

Promoter Hypermethylation of *GADD45G* AND $P16^{(INK4a)}$ Genes has Role in Chronic Myeloid Leukemia Progression

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Chronic myeloid leukemia is clonal haematopoetic stem cell disorder characterized by philadelphia chromosome. CML has chronic progressive course and progress from chronic to accelerated phase and finally blast crisis phase. Progression of CML is due to many factors and one of them is inactivation of tumor suppression gene eg. $P16^{(INK4a)}$, *GADD45G*.

The present study was carried out to detect *GADD45G and* $P16^{(INK4a)}$ genes promoter hypermethylation in chronic myeloid leukemia patients and healthy subjects and compare the results of *GADD45G and* $P16^{(INK4a)}$ genes promoter hypermethylation in chronic myeloid leukemia patients and healthy subjects. Further, the association of *GADD45G* and $P16^{(INK4a)}$ genes promoter hypermethylation with clinical phases(chronic, accelerated and blast crisis) will also be carried out.

The study was done in 30 cases of CML patients [includes 10 patients of each phase of CML (Chronic Phase, Accelerated Phase, Blast Crisis)] and 30 healthy subjects controls. Methylation status of *GADD45G* and $P16^{(INK4a)}$ genes were evaluated by Methylation specific-PCR. Frequency of promoter hypermethylation of $P16^{(INK4a)}$and *GADD45G* genes in whole blood of CML patients was observed to be 46.7% and 40% respectively.

We observed statistically significant difference in methylation status of genes in whole blood DNA with cases (30 CML patients) and controls (30 healthy controls) (p-value <0.0001 by Fisher exact test for both genes). No significant association was found between hypermethylation of $P16^{(INK4a)}$and *GADD45G* genes with age, sex and thrombocytopenia status. We found that there is significant association between methylation status of $P16^{(INK4a)}$ with clinical stages of CML patients (p-value = 0.03 by Fisher exact test). We didn't found any association between methylation status of *GADD45G* with clinical stages of CML patients (p-value = 0.89 by Fisher exact test).

Our results suggest that $P16^{(INK4a)}$ is a primary target for inactivation by promoter methylation which has role in disease progression of CML patients.

O36

Effect of L/B/K Alkaline Phosphatase Transcript on Renal Cell Carcinoma Cell Lines: Plausible Role in Tumorigenesis

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Renal cell carcinoma (RCC) is the most common kidney cancer in adults. Although several genes have been found to be

involved in carcinogenesis of RCC, more great efforts are needed to identify new genes which are responsible for the process. Alkaline phosphatase (ALP) is a marker enzyme of brush border membrane of proximal tubular cells. Our previous studies showed a significant decreased activity of Liver/Bone/Kidney (L/B/K) alkaline phosphatase in RCC. In the present study, we explored the plausible effect of decreased activity of ALP in RCC carcinogenesis. The L/B/K ALP cDNA were transiently transfected into ACHN and A498 cells and its effect on cell viability of ACHN as well as A498 cells were determined by MTT assay. The cell migration was determined by scratch assay and apoptosis was determined by FACS analysis with Annexin/PI staining kit. RCC cell lines (ACHN and A498) were transfected with full length L/B/K cDNA that showed decreased migratory property as well as viability of these cells as compared with controls ($P < 0.000$). Further, L/B/K ALP cDNA transfected cells (ACHN and A498) showed significant increased apoptosis as compared to control ($P < 0.000$). These findings suggest the new role of L/B/K ALP in cell viability and apoptosis and involvement in RCC tumorigenesis.

O37

Serum Human Epidermal Growth Factor Receptor-2 (sHER-2/neu) in Breast Cancer Patients and its Comparison with Clinicopathological Parameters

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Breast cancer patients with HER-2/neu amplification or overexpression are eligible for treatment with transtuzumab (Herceptin). In clinical practice, over-expression of HER2/neu is routinely identified using Immunohistochemistry (IHC) and Fluorescence in situ Hybridization (FISH), both of which are invasive approaches requiring tissue samples.

The sECD/HER-2/neu fragment from the surface of breast cancer cells once shed into the blood of individuals can be quantified using commercially available enzyme-linked immunosorbent assays, making it a useful breast cancer biomarker.

The present study was carried out to compare the levels of sHER2/neu with IHC and find the association of sHER2/neu with clinicopathological parameters.

75 histologically confirmed female breast cancer patients in the age group of 26-75 years were recruited. Information on patient's age, menopausal status, disease stage, tissue HER2/neu status, estrogen receptor (ER), progesterone receptor (PR) status, clinical nodes were noted from the case files. sHER2/neu levels were measured by Ray-Bio Human ErbB2 Elisa kit. Cut-off value of >=15 ng/ml ws used to define elevated sHER2/neu.

Mean age of the cases were 47.97 +/- 12.67 years. 70.6% (53/75) of the patients were sHER2/neu positive and 53.3% (40/75) were IHC positive. There was a significant correlation found between sHER2/neu and IHC ($p = 0.05$). Statistically significant association was found between sHER2/neu and histological grade ($p = 0.043$) and clinical stage ($p = 0.05$). No statistically significant association was found between sHER2/neu and age, menopausal status, lymph node status, histological tumor type, ER status, PR status and molecular type.

Presently, sHER2/neu by ELISA cannot replace IHC/FISH assays but will definitively complement the tissue assays to offer a real time picture of HER2/neu status. sHER2/neu testing may be a useful tumor marker for monitoring breast cancer patients, even in those with negative IHC.

O38

Evaluation of Anticancer Activity of Indian Medicinal Plants

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Natural products have been a key source in the development of various therapeutics agents due to diverse ethnomedical properties. However, researches on natural medicinal plants have lately undergone extensive growth owing to advances in drug discovery, isolation techniques, synthetic methods, physiochemical measurements and new concepts. India has vast diversity of natural products but only few have been evaluated for anticancer properties.

In our ongoing screening program of anticancer evaluation of natural products we have screened some ethnomedical plants for anticancer potential. In this study we represent the anticancer potential of seeds of *Crotalaria juncea* L. and leaves of *Nerium oleander* L.

We have performed in vitro cytotoxicity via MTT assay for antiproliferation study, Hoechst staining for morphological apoptosis and Colony formation assay of different fractions against Human Breast cancer MDA-MB-231 cells.

Results obtained revealed that different fractions exhibited dose and time-dependent killing capabilities of breast cancer cells. Moreover, cell death caused by different fractions was via apoptosis.

In conclusion, different plant fractions showed particularly strong anticancer capabilities since they inhibited proliferation of breast cancer cells. Therefore, our study suggests that plant fractions have promising anticancer properties and that they may be considered as a source for the isolation of their active principles from enriched bioassay-guided fractions.

P001

Oxygen-18 Isotope of Breath CO_2 Linking to Erythrocytes Carbonic Anhydrase Activity: A Biomarker for Pre-Diabetes and Type 2 Diabetes

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Carbonic anhydrase (CA), a well-characterized pH-regulatory metalloenzyme found in most tissues including human red blood cell (RBC), rapidly catalyses the hydration of carbon dioxide (CO_2) to form bicarbonate (HCO_3^-) and the reversible dehydration. It is associated with oxygen-18 (^{18}O) isotopic fractionations of CO_2 during respiration. The aim of the present study was to investigate the role of CA activity in RBC in the pathogenesis of early stage (i.e. pre-diabetes) and type 2 diabetes and thereafter to find out the potential link between CA activity and ^{18}O-isotopic exchange of breath CO_2 for pre-diabetes and type 2 diabetes. Pre-and post-dose breath and blood samples were collected simultaneously after administration of 75-gm normal glucose dissolved in 150-mL water. The breath samples were analysed by a laser based high-resolution carbon dioxide isotope analyser to measure the carbon dioxide isotopes ($^{12}C^{16}O^{16}O$, $^{13}C^{16}O^{16}O$ and $^{12}C^{16}O^{18}O$). Blood samples were utilized to measure the esterase activity of carbonic anhydrase spectrophotometrically. We found that the pre-diabetes (PD) and type 2 diabetes (T2D) exhibited isotopic enrichments of ^{18}O-isotope in breath CO_2, while a marked depletion of ^{18}O in CO_2 was manifested in the non-diabetic controls (NDC), when compared with their basal values. However, post-dose CA activity in both T2D and PD was markedly enhanced, whereas NDC exhibited a considerable reduction in post-dose CA activity with respect to basal CA activities. From receiver operating characteristic curve analysis (ROC), an optimal diagnostic cut-off point was determined to be $\delta^{18}_{DOB}O‰ > 2.77‰$ and $\delta^{18}_{DOB}O‰ < -1.14‰$ for screening individuals with T2D and NDC respectively, whereas subjects with $2.77 \geq \delta^{18}_{DOB}O‰ \geq -1.14$ were suggested to be PD and these corresponded to the diagnostic sensitivity and specificity of ~95% and ~91%, respectively. Our findings suggest that the breath $C^{18}O^{16}O$ isotopes (oxygen-18 isotope of breath CO_2) regulated by the CA activity may be a potential biomarker for non-invasive assessment of T2D, and thus may open a new method for treating T2D.

P002

Assessment of Inflammatory Markers IL-6 and hs- CRP Among type 2 Diabetes Mellitus Patients Attending Central Referral Hospital, Gangtok

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Diabetes mellitus is a multi-factorial disorder of metabolism characterized by chronic hyperglycemia, with heterogeneous etiologies. Inflammation is emerging as a cause and inflammatory markers like IL-6 and has-CRP are suggested to have a possible role in the pathogenesis and development of complications associated with type 2 diabetes mellitus. To evaluate the status of inflammatory activity in type 2 diabetes mellitus (T2DM) patients in comparison to healthy controls. This study was undertaken in 150 subjects visiting Central Referral Hospital, Bangkok in the age group of 35 to 65 years, between September 2014 - August 2015. The diagnosed cases ($n = 100$) of T2DM were compared with healthy controls ($n = 50$). Prior institutional ethical clearance and informed consent were obtained. Height and weight were measured and Body mass index (BMI) was calculated. Serum hs-CRP and IL6 were estimated by ELISA, HbA1c assay was done by particle enhanced immuno turbidimetric method, fasting and post prandial blood sugar were estimated by GOD POD method in Erba EM200. The mean BMI among patients of T2DM was 24.13 ± 3.17 kg/m^2. Serum IL-6 (2.62 ± 1.92 pg/ml) was higher among T2DM patients than healthy controls (0.88 ± 0.53 pg/ml; $p < 0.001$). Similarly hs-CRP was significantly higher among cases (6.85 ± 5.29 mg/l) than controls (1.83 ± 1.2 mg/l) ($p < 0.001$). BMI was not significantly different in the two groups but serum levels of IL-6 and hs-CRP are higher in T2DM patients as compared to healthy controls, suggesting a possible role of increased inflammatory activity in Type 2Diabetes Mellitus.

P003

Comparative Evaluation of the Effects of Yoga and Exercise in Perimenopausal Women with Metabolic Syndrome

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Metabolic syndrome is associated mainly with cardiovascular diseases and type 2 diabetes, and is a growing problem worldwide. People with metabolic syndrome are about twice as likely to develop these disorders compared to subjects without metabolic syndrome. Regular physical activity either yoga or exercise is one of the most important modes of mitigating the effects of risk factors of metabolic syndrome. The purpose of this study was to analyse the effects of yoga and exercise on the anthropometric and cardiovascular indices of metabolic syndrome in perimenopausal women. Sixty four women aged 48.34 ± 4.63 years with perimenopausal symptoms were randomly assigned to either a yoga group ($n = 30$) or to an exercise group ($n = 34$) considering inclusion and exclusion criteria set for the study. The participants were checked for anthropometric parameters, glycemic index and serum lipid profile measurements before and after 12-weeks of yoga or exercise intervention. Body weight and body mass index had significantly decreased ($P < 0.001$) in yoga group. Waist and hip circumference was significantly decreased ($P < 0.001$) in both yoga and exercise group. High-density lipoprotein cholesterol had significantly increased ($P < 0.05$) in yoga group. Total cholesterol, triglyceride, low-density lipoprotein cholesterol and Glycated Hb had significantly decreased ($P < 0.05$) in

both yoga and exercise group. Systolic blood pressure in the yoga group and diastolic blood pressure in both the groups were significantly decreased ($P < 0.05$) after the intervention. The findings of the study indicate that yoga and exercise have significant health benefits in perimenopausal women. Consequently it can be effectively used in reducing the risk of cardiovascular disease and type 2 diabetes in perimenopausal women.

P004

Is Succinyl Acetone a Common Metabolic Link Between Tyrosinemia and Alkaptonuria?

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Succinylacetone is an abnormal metabolite found in Tyrosinemia –I (HT) due to the primary deficiency of fumaryl acetoacetate hydrolase. Interestingly this metabolite is not only specific to this autosomal recessive disease, but is also found in one of the rare and first discovered inherited disorder alkaptonuria. Though the metabolic pathway is same for both the disorders, the primary defects involved in both of these are distinct. Hence, this study suggests that presence of SA shouldn't be misdiagnosed for tyrosinemia. The objective of this study was to demonstrate the presence of SuccinylAcetone in alkaptonuria and tyrosinemia. Succinylacetone was assayed by spectrophotometry and the organic acid profile determined by TLC. Spectrophotometric assay showed the presence of succinylacetone by the inhibition of δ-aminolevulinate dehydratase (ALA-D) in both the disorders, but comparatively less in alkaptonuria positive samples. However qualitative measurement by TLC also showed the presence of SA spot, thereby confirming the presence of succinylacetone in alkaptonuria also. As the presence of succinylacetone was confirmed by the methods mentioned, we hereby conclude that succinylacetone isn't a specific marker for hereditary tyrosinemia – I as it can lead to misdiagnosis for the same, so alkaptonuria samples should be followed up and diagnosed suitably with consecutive specific panel of test.

P005

Implications of Advanced Glycation end Products, Receptor for Advanced Glycation end Products, Soluble Receptor for Advanced Glycation end Products, and High-Sensitivity C-Reactive Protein (hs-CRP) in the Pathophysiology of Diabetes Mellitus with Hypertension

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Advanced Glycation End Products (AGEs) and their receptors are strongly implicated in the development of diabetes complications. Soluble receptor for AGE (sRAGE) may entangle to counteract the detrimental effects of receptor for AGE (RAGE). Therefore, the detailed study was conducted to evaluate the levels of high-sensitivity C-reactive protein (hs-CRP), chronic myeloid leukaemia (CML), Pentosidine, RAGE, s-RAGE to understand the molecular pathophysiology of diabetes associated hypertension. The objective of this study was to evaluate the blood levels of AGE, RAGE, sRAGE and hs CRP in patients with diabetes associated hypertension. Thirty patients each with type-2 diabetes with hypertension and type-2 diabetes without hypertension were enrolled in the study. CML, Pentosidine, RAGE and sRAGE, hs CRP levels were measured using the commercially available kits as per the manufacturer's instructions. The results show that increased levels of CML, Pentosidine, low levels of s-RAGE, increased levels of RAGE and increased levels of hs-CRP were observed in diabetes associated hypertension group in comparison to controls. However in the follow up patients we observed raised levels of sRAGE, reduced levels of RAGE, hs-CRP, CML and Pentosidine as compared to diabetes, hypertention and diabetes associated hypertention group. However the levels were not same as that found in control group. It suggests that reduced levels of RAGE, hs-CRP and AGE's (CML and Pentosidine) correlates with improvement in disease process. We conclude an association between lower sRAGE levels in diabetes and diabetes associated hypertension group and sRAGE may be a useful biomarker for the prediction of diabetes related hypertension. Taken together these findings indicate that low levels of sRAGE are a marker of future chronic disease risk and mortality in the community and may represent an inflammatory state.

P006

MMP-2, MMP-9, TIMP-1 and TIMP-2 Levels in Patients with Recurrent Pregnancy Loss

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The American Society for Reproductive Medicine has defined recurrent pregnancy loss (RPL) as two or more failed a fetus up to 20 weeks of pregnancy and weighing up to 500 g. The exact etiologies for the recurrent pregnancy losses have not been clearly explicated, and time and again remain undefined. Trophoblast cell invasion into the maternal endometrium plays a crucial role during human embryo implantation and placentation. This invasion is facilitated by the activity of matrix metalloproteinases, which are regulated by tissue inhibitors of MMPs (TIMPs). The objective of the study was to investigate the serum levels of MMP-9, MMP-2/TIMP-2 complex, TIMP-1 and TIMP-2 in patients with recurrent pregnancy loss, correlate with healthy women as well as women with 1st trimester of pregnancy. Zymographic analysis showed compares the serum levels of MMP-9, MMP-2/TIMP-2 complex, TIMP-1 and TIMP-2 in 50 patients with recurrent pregnancy loss and compared with that of 40 healthy volunteers as well as women with 1st trimester of pregnancy. MMP-9 was markedly elevated in missed abortions, as was MMP-2/TIMP-2 complex. However, the serum levels of TIMP-1 and TIMP-2 were markedly elevated in control groups. Human placentation is mediated by fetal trophoblastic cells that invade the maternal uterine endometrium. Trophoblast invasion requires a precisely regulated secretion of specific proteolytic enzymes able to degrade the endometrial basement membrane and extracellular matrix. The elevated levels of MMP-9 and MMP-2/ TIMP-2 complex may play a role in spontaneous termination of pregnancy.

P007

A Clinical Revise to Evaluate Antioxidant Activities of *Tinosporacordifolia*in Management of Type – 2 Diabetes Mellitus

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Tinosporacordifolia (Menispermaceae) is widely used in ayurvedic medicine as a remedy for metabolic disorders. However, its anti-oxidant activities are not well studied. The objective of the study was to explore the anti-oxidant activities of *Tinosporacordifolia* (Menispermaceae) in type 2 diabetic patients. In this study clinical trial was conducted on type 2 diabetic patients attending diabetes OPD, Kaya ChikitshaVibhag, Ayurvedic Chikitsha Mahavidyalaya, Touriya Ganj, Lucknow as per guide line of ethics. All biochemical assays were done by standard kit methods. A marked increase in the levels lipid peroxide while decrease in the levels of reduced glutathione, superoxide dismutase and catalase were noted in type 2 diabetic patients compared to healthy controls. However, oral administration of powdered stems (50 mg/kg body weight, p. o.) for 15 days reversed these effects. Results of this study lead to research and development of anti oxidant drug from *Tinosporacordifolia*.

P008

Patterns of Biomarkers in Cord Blood during Pregnancy and Preeclampsia

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During pregnancy inflammatory, metabolic and immunologic disorders that affect differently the fetus are known. These could be early disorders: abortion, intrauterine growth retardation, low birth weight and neonatal death; or late disorders: cardiovascular and metabolic disease in adults. The objective was to analyse different biochemical parameters in maternal venous blood and newborn's umbilical cord blood (UCB) from healthy and pathological mothers for early detection of future perinatal complications. Maternal and cord blood homocysteine, folate, B_{12}, heme oxygenase-1 (HO-1), endoglin, leptin, cholinesterase, IGF-1, Apo A lipoproteins, TSH, fT_3, fT_4 were investigated in maternal sera and in the venous umbilical cord sera of the corresponding new-borns of fifty women with preeclampsia (group II) and fifty normotensive pregnant women (group I). Homocysteine, Folic acid, Vitamin B_{12}, Apo AI and II, TSH, fT_3, fT_4levels were estimated in serum by competitive immunoassay using direct chemiluminiscence technology. Hemeoxygenase-1 (HO-1), endoglin, leptin, cholinesterase, IGF-1 were analysed by ELISA. Results of various parameters in maternal and cord blood of both the groups will be presented and discussed. Findings of present study suggest that biochemical alterations of fetuses occur which can result in endothelial cell dysfunction. Modifications of uterine environment in terms of thyroxine and folate and vitamin B12 supplementation can be of help in lowering the damaging effects of homocysteine on vascular endothelium, development of preeclampsia and future risk of cardiovascular risk.

P009

Emerging Role of Vitamin D in Metabolic Syndrome

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Vitamin-D is a hormone which is responsible for mainly maintenance of our body and skeletal integrity. Recently a is rise in the number of Vit-D deficiency is noticed worldwide which is due to inadequate exposure to UV rays, has drawn the world's attention on the extra skeletal function of this golden vitamin. The steady rise in the number of Type II DM, not only in the India but throughout world has drawn the attention towards connection of Vit-D deficiency with Type II DM and has shown that there is a positive correlation between Vit-D deficiency and Type II DM. The discovery of vit-D receptors expressed in almost all the cells of the body which includes immune, vascular, myocardial cells, pancreatic beta cells, neurons and osteoblasts suggest an involvement of Vit-D mediated effects on Metabolic Syndrome (MS). Very few studies has been done in this regard. Many clinical trials have suggested improvement in the insulin secretion and sensitivity with supplementation of Vit-D in Pre-diabetic individuals. Correcting Vit-D level can prevent or even delay the onset of diabetes as well as MS and has shown to be a promising field to explore. Longitudnal studies may prove to be beneficial for making a strategy for supplementation of this vitamin which might prevent or delay the process of development of Type II DM which is interlinked with MS and can save the mankind.

P010

Expediency of Markers for Early Detection of Acute Kidney Injury Sequela to Type 2 Diabetes Mellitus

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Diabetes is a metabolic disorder leading to micro and macro vascular complications. Acute Kidney Injury (AKI) is one of the acute complications seen in diabetes, associated with increased morbidity and mortality. Estimation of Cystatin C, traditional markers, inflammatory and endothelial cell activation markers can identify subjects who were at increased risk for future AKI after diabetes. The

objectives of the study were to estimate and compare anthropometric, inflammatory, oxidative stress and other biochemical parameters in clinically proven healthy controls (Group-I), T2DM without AKI (Group-II) and T2DM with AKI (Group-III) and to correlate Cystatin C and creatinine in Groups II and III with anthropometric and biochemical parameters to know the severity of kidney injury as well as to make ROC analysis of Cystatin C, Creatinine and eGFR to predict better biomarker for AKI. Total of 210 subjects, having 70 subjects in each group between age group of 45–75 years were enrolled in our study. Anthropometric and biochemical parameters were measured and estimated by using standard methods. Group III showed significant increase in anthropometric and biochemical parameters except for vitamin C and eGFR. BMI, HbA1c, eGFR, ACR, Vitamin C, MDA and nitric oxide showed significant positive correlation with Cystatin C and creatinine in Groups II and III. ROC analysis showed Cystatin C as the better biomarker to detect early renal damage. We conclude that elevated levels of biomarker Cystatin C, serum creatinine and ACR are predictors of AKI in the setting of diabetes. Baseline inflammatory and endothelial activation markers may also be useful for predicting future risk of AKI in diabetes mellitus.

P011

Association of Homa-IR and Small Dense LDL to Determine IR in Young Obese Type 2 Diabetic Subjects in Both Sex

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The formation of sdLDL is increased in the presence of insulin resistance and dyslipidemia in diabetic subjects. Type 2 diabetes mellitus now-a-days, also known as low grade chronic inflammatory disease especially when it is associated with obesity. Obesity-induced insulin resistance is associated with increased lipid concentrations in insulin-responsive tissues, normal glucose tolerant people with enlarged subcutaneous abdominal adipocytes and elevated levels of FFA which are at increased risk of developing T2DM. IR also appears to play an important role in the pathogenesis of this type of dyslipidemia. Our study aims to investigate the association of Homa-IR and small dense LDL in Young obese type II diabetic subjects in both sexes. This study was carried out in Department of Biochemistry, G.R.Medical College, Gwalior and part of the work in Laboratory Medicine, Department of Biochemistry, Medanta-The Medicity Hospital, Gurgaon. 100 known type 2 diabetic subjects (M & F) with age 24–40 years were included in the study and 100 were healthy control, age matched having normal body mass index (BMI). Statistical analysis was done using multiple regressions which has proved the above relationship between IR and obesity. sdLDL was positively associated with Homa-IR ($p < 0.05$), TG ($p < 0.01$), LDL-C ($p < 0.01$), VLDL-C ($p < 0.01$) and TC/HDL-C ratio ($p < 0.01$) while, negatively associated with cholesterol level ($p < 0.01$) and HDL-C ($p = NS$) in young obese group (both sex). The study shows that, obesity in young age is main precipitating factor for insulin resistance and high insulin resistance block the way for the utilization of glucose and thus, precipitates the diabetes mellitus, hence, these findings justify that obesity in type 2 diabetics is associated with hyperinsulinemia, hyperlipidaemia and glycemic conditions which are more prone for CVDs in young diabetic population.

P012

Association of Serum Nitric Oxide and Serum Xanthine Oxidase with Cardiac Biomarkers in Myocardial Infarction Patients

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Myocardial Infarction (MI) results from the reduction of coronary blood supply to the myocardial tissue. Serum Troponin T and NT-Pro-BNP along with lipid profile estimations are being used to confirm the diagnosis of MI. In this study we are looking for alternative markers which can help in diagnosing MI. The objective was to find out the association of serum Nitric oxide (NO) and serum Xanthine oxidase (XO) with cardiac biomarkers in MI patients. Two ml of blood sample is collected from MI patients and the serum is used to estimate NO and XO by colorimetric method. The results showed negative weak correlation of NO and XO with Troponin T and NT-Pro-BNP in MI Patients. The levels of NO and XO where found to be decreased in patients with high Troponin T and NT-Pro-BNP levels. NO is an important mediator of vasodilation in blood vessels. It is once synthesized by eNOS, it results in phosphorylation of several proteins that cause smooth muscle relaxation. In our study we found the decreased level of NO leading to vasoconstriction of already atherosclerotic blood vessels. We also found decreased level of XO which indicates decreased production of ROS formation in MI patients from XO but ROS generation may be due to other mechanisms like Lipid Peroxidation.

P013

Study of Glycated Hb in Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is the commonest cause of ovulatory infertility. It is the most common endocrinological disorder among women in reproductive age group and the study of HbA1C in PCOD help us to understand the risk factor associated with type 2 Diabetes. Our aim was to study the level of glycated Hb in PCOD patients. This cross sectional study was carried out among 50 PCOD patient attending infertility OPD of SRM Medical College Hospital & Research Centre, 50 healthy individuals were included as controls. Fasting Blood sample were collected from both groups. HbA1C was estimated by HPLC method using D10analyser. PCOD was examined based on Rotterdam criteria: Anovulation or Irregular Periods, Hyper-androgenism like adult acne, hirsutism (a male pattern of body or facial hair), or hair loss (androgenic alopecia) and ultrasound examination to identify small cysts in ovaries. All the statistical analysis was performed using statistical package SPSS. The mean values of HbA1c, FBS were significantly high in patients with PCOS compared to control ($p < 0.0001$). A regular monitoring of HbA1c act as a tool for diagnosing type 2 Diabetes, insulin resistance in PCOD patient.

P014

Visceral Adiposity Index and Leptin Levels in Overweight Smoking Individuals

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Cigarette smoking is one of the major risk factor for cardiovascular disease. Cigarette smoke is composed of many carcinogenic chemicals that trigger inflammatory state. The adipocytokine like Leptin has been implicated in the regulation of body weight and energy expenditure. Leptin is identified to play an important role in obesity and inflammation. The main objective of this study is to correlate visceral adiposity index and serum Leptin levels in male overweight smokers and overweight non-smokers. Subjects with waist circumference more than $\geq$ 95 cm were recruited from Kattankulathur area. We measured Leptin concentration (DRG Sandwich – ELISA) and blood lipid levels in 30 middle aged male smokers and 30 non-smokers. Visceral adiposity Index and body fat % was calculated. Mean of serum Leptin showed a statistically significant elevation in the overweight smokers as compared to overweight non-smokers ($p < 0.05$). Leptin levels significantly correlated with body fat %, Visceral adiposity Index and duration of smoking. Our study showed a significant strong negative correlation with HDLc and positive correlation with Non-HDLc (TC - HDLc) in chronic male smokers. Thus, male chronic overweight smokers have a higher leptin levels. Smoking not only triggers immune system but induces a decrease in hypothalamic sensitivity to leptin and thereby resulting in leptin resistance. Visceral adiposity index and smoking justifies the augmented cardiovascular risk in the overweight smokers.

P015

Lipid Profile of Women During Different Modes of Delivery

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As pregnancy advances, hormones induced hyperlipidemia make women more atherogenic. This physiological adaptation serves the increased energy needs of mother, supply steroid hormone precursor for the placenta and provides cholesterol and essential fatty acids for the fetus. Maternal hyperlipidemia is well reported but much work has not been done regarding the lipid levels in different modes of delivery. The objective of the study was to assess the maternal lipid levels during vaginal delivery and caesarean section. Fifty non-pregnant women served as control (Group I). 200 pregnant women, admitted in Labor room for delivery were grouped (50 each) as Group II-at term, Group III- vaginal delivery, Group IV- operative delivery, Group V- 48 hours postpartum. Blood sample was drawn at admission if not in labor, during delivery and within 48 hours postpartum. Total lipids, cholesterol, HDL-cholesterol and triglycerides were estimated on Auto analyzer Hitachi-911 Free Fatty Acids were estimated by Lowry and Tinsley method. Serum lipid levels increased significantly at term from the non-pregnant levels ($p < 0.001$). The increase in triglyceride level was maximum. during vaginal delivery. Lipid levels were comparable at term non significantly but are higher than in controls significantly. Total lipids, total cholesterol, FFA were higher but HDL and TG had no significant difference between groups II and III. During caesarean section, lipids levels were lower when compared with those of vaginal delivery and at term, but significantly higher than control ($p < 0.01$). FFA, HDL and TG levels were higher during vaginal delivery than in caesarean section, but total cholesterol remained same. During postpartum, lipid levels decreased abruptly but were still higher than control. All lipids levels were higher in vaginal delivery cases than during caesarean section. This may be due to labor induced stress, which increases glucocorticoids resulting in mobilization of lipids from fat stores, thus provide fatty acids for maternal energy.

P016

Association Between Adiponectin and Insulin Resistance in Diabetic Urolithiasis

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The prevalence of urolithiasis is increasing worldwide. Diabetes mellitus is characterized by insulin resistance which increases the risk of kidney stone formation. Adiponectin is an insulin-sensitizing and anti-inflammatory cytokine which is known to improve glucose tolerance and insulin resistance in humans. The association of insulin and adiponectin with kidney stones is not clear. Hence, the present study was undertaken to assess the serum levels of adiponectin and insulin resistance in diabetes mellitus patients with urolithiasis in comparison to those without urolithiasis. The study involved two groups. Group A consisted of 30 diabetes mellitus patients with urolithiasis and group B consisted of 30 diabetes mellitus patients without urolithiasis. Biochemical parameters studied were serum adiponectin, insulin, glucose, urea, creatinine, Homeostasis model assessment of insulin resistance (HOMA-IR), 24 hrs urinary calcium and phosphate. The results showed that serum adiponectin level was significantly increased in diabetic urolithiasis cases (Group -A) when compared to the control group (Group -B). The levels of 24 hours urine calcium and phosphorus were also significantly increased in the diabetic urolithiasis cases. There was no significant difference in serum insulin and HOMA-IR between the two groups. A negative correlation was seen between serum adiponectin and insulin among the cases (r value $= -0.368$ and $p = 0.045$). We report an increase of serum adiponectin levels in diabetic patients with urolithiasis who also have a higher BMI levels.

P017

High Body Mass Index May Not Predict Metabolic Syndrome

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Body Mass Index (BMI) and metabolic syndrome (MS) increases the risk for cardiovascular disease and type 2 diabetes mellitus (T2DM) independently and synergistically. The objective was to evaluate the role of a high BMI (overweight and obese) in predicting MS. A total of 195 patient's $\geq$ 20 yrs. of age who visited the central laboratory of Central Referral Hospital, Sikkim with a requisition form for fasting blood sugar (FBS) and lipid profile who willingly participated in the study. Pregnant ladies, smokers and anyone under medication for the previous one month for conditions other than T2DM, hypertension and lipid abnormality were excluded. Participants were evaluated for BMI and for presence or absence of MS (International Diabetes Federation, IDF). A logistic regression was conducted to ascertain the effects of a high BMI: overweight (25-29.9 kg/m^2), obese I (30–34.9 kg/m^2), obese II (35 – 39.9 kg/m^2) and extreme obese ($\geq$ 40 kg/m^2) on the likelihood of having metabolic syndrome. Of the 195 participants MS was present in 38 / 82 (38.6%) normal BMI and 63/107 (58.87 %) high BMI. Patients with high BMI (n = 107) were 0.873 times less likely to have MS, OR = 0.873, CI (0.769 - 0.992) after adjusting for age and gender ($p < 0.05$). Having a high BMI does not reflect the risk of developing MS by itself. MS was seen in individuals with normal weight thereby supporting the need to consider other indices of obesity while screening for metabolic syndrome.

P018

Hypoglycemic Effect of Fraction II Obtained from Extract of *Ficus infectoria* leaves in Streptozotocin Diabetic Rats

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Ficus infectoria is a plant of 'Moraceae' family known as 'White Fig' in English, 'Pilkhan' in Hindi. It is a plant of great medicinal value. The objective was to assess the hypoglycemic activity of a purified fraction obtained from extract of *Ficus infectoria* leaves in streptozotocin diabetic rats. Aqueous extract of leaves was applied to Silica gel column and eluted with methanol and ethyl acetate (65:35). This afforded three fractions – Fraction FI, FII and FIII. Dried fractions suspended in water were fed to animals. Rats were made diabetic by i.p. injection of streptozotocin (45 mg/kg) and divided into six groups of six rats each as follows: Group I- Healthy control, Group II – Diabetic control, Group III, IV and V diabetic rats treated with FI, FII and FIII respectively (20 mg/kg bwt) Group VI-received glibenclamide (0.5 mg/kg) orally once daily for one month. FII exhibited significant hypoglycemic activity, FII and FIII were not effective. Treatment with FII decreased Fasting blood glucose by 54% and postprandial blood glucose by 53% ($p < 0.0001$). It also decreased Glycosylated hemoglobin significantly ($p < 0.0001$), improved lipid profile; -Decreased Total cholesterol, Triacylglycerol, LDL-C + VLDL-C while HDL-C increased significantly ($p < 0.001$). There was significantly increased fasting serum insulin, C-peptide and liver and muscle glycogen ($p < 0.001$). Results were comparable to result obtained with standard drug glibenclamide. Present study indicate fraction II obtained from *Ficus infectoria* leaves have potent hypoglycemic and hypolipidemic effect.

P019

Correlation of High Sensitivity C-Reactive Protein (Hs-Crp) With Cardiovascular Risk Variables in Impaired Fasting Glycemic Subjects

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Cardiovascular diseases are the major cause of morbidity and mortality in patients with Diabetes Mellitus. High sensitivity C-Reactive Protein (hs-CRP) is a nonspecific inflammatory marker that predicts cardiovascular risk in the subjects. Impaired fasting Glycemia is a condition in which the fasting plasma glucose is between 110 and 126 mg/dl with normal two-hour post glucose value. The study aimed to estimate serum High sensitivity C-Reactive Protein (hs-CRP) levels and assess the cardiovascular risk in the subjects with impaired fasting glycemia. This study is a cross-sectional study involving 100 patients attending the Medicine OPD at SRMMCH & RC. Group-1 includes 100 euglycemic subjects. Group-2 includes 100 Impaired Fasting glycemic subjects. After overnight fasting, blood samples collected and estimated for High sensitivity C-Reactive Protein (hs-CRP), fasting blood glucose, and total cholesterol, HDL-C, LDL-C and Triglycerides in AU- 400 auto-analyser. Statistical analyses by statistical package SPSS 21.0 and Pearson's correlation analyses determine the relationship between hs-CRP with other cardiovascular risk variables. High sensitivity CRP, an inflammatory marker is elevated in subjects with altered glycemic status. A statistically significant correlation between hs-CRP and fasting blood glucose, LDL-C, Non-HDL-C and Triglycerides justifies the Cardiovascular risk. Thus hs-CRP predicts the CV risk in prediabetic subjects.

P020

Interleukin-6: Cardiovascular Disease Risk Marker in Prediabetes

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Prediabetes is associated with dysglycemia, endothelial dysfunction, obesity and inflammation, placing them at an increased risk of cardiovascular events. The present study aimed to investigate the risk of cardiovascular disease (CVD) associated with prediabetes by estimation of serum interleukin-6(IL-6) and its correlation with fasting plasma glucose (FPG) and anthropometric measurements. A cross sectional study was conducted over a period of one year in a tertiary care hospital, Mangalore. Eighty subjects were categorised into prediabetes and healthy controls based on their FPG values. Anthropometric data (weight, BMI, waist circumference (WC), hip circumference (HC) and waist-to-hip ratio (WHR)) from all subjects were recorded. IL-6 was estimated in serum sample. The mean anthropometric measurements and IL-6 was found to be significantly higher ($p < 0.05$) in prediabetes group. IL-6 had no significant correlation with FPG (r-0.227) in the prediabetes group. IL-6 also showed a positive correlation with BMI(r-0.339), WC(r-484) and WHR(r-0.430) in prediabetes group. This study suggests that

prediabetes is associated with central adiposity and have increased levels of IL-6.

P021

Evaluation of Metabolic Syndrome and Vitamin D Receptor Gene Polymorphism in Male Factor Infertility

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Male factor infertility (MFI) and Metabolic Syndrome (MetSyn) is a growing health issue in the society in recent years. MFI represents one such perturbation in some male patients with MetSyn. The Vitamin D Receptor gene (VDR) is expressed in many tissues including reproductive organs. The study objective was to find out the association of MetSyn and insulin resistance with MFI; association of MetSyn with VDR gene (*FokI*, rs 2228570; C>T) polymorphism in MFI; and also the association of VDR gene polymorphism with MFI. This hospital based case control study was conducted at Maulana Azad Medical College, New Delhi. Total 104 subjects (50 cases, 54 age and sex matched controls) fulfilling inclusion and exclusion criteria were included in the study. Fasting plasma glucose, serum triglyceride and HDL-C estimation were done by clinical chemistry analyser by standard methods. Serum insulin and vitamin D was estimated by electro chemiluminescence immunoassay and VDR gene polymorphism by RFLP-PCR. All statistical analysis was done with SPSS 18. The results showed that there was significant difference of HOMA-IR and occurrence of MetSyn between MFI cases and controls. Association of VDR gene polymorphism with MFI was present and the serum level of vitamin D was also decreased in MFI cases than in controls, p value <0.05. Association of serum vitamin D level and MetSyn was present. Occurrence of MetSyn and its components was higher in MFI cases than in controls. VDR gene polymorphism increases the risk of MFI.

P022

Relationship Between Sialic Acid and Markers of Type II Diabetes Mellitus in Blood and Saliva

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Worldwide, the prevalence of diabetes mellitus has risen at alarming rates in the past few decades. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. Studies suggest that inflammation plays a role in the pathogenesis of the glucose disorder in adults. Type 2 diabetes mellitus and atherosclerotic cardiovascular disease have common antecedents. Markers of inflammation predict cardiovascular disease and are raised in patients with type 2 diabetes. Circulating serum sialic acid, an inflammatory marker has recently been shown to be a strong predictor of cardiovascular mortality, and obesity- related diseases. Researchers have found that some organic and inorganic components of saliva are modified in diabetic patients. The aim of this study was to study the relationship between sialic acid and markers of Type II Diabetes Mellitus, in blood and saliva. The study was an observational case control study. It was carried at the Advance Research Lab of Institute of Technology and Science – Centre for Dental Studies and Research (ITS-CDSR), Muradnagar, Ghaziabad in collaboration with Avantika Hospital, Indirapuram, Ghaziabad over a period of one year (January 2014 to April 2015). The study comprised of the case group including 42 Type II DM patients and control group included 41 non diabetic patients between the ages of 35 to 70 years. The study subjects were carefully selected after screening for the inclusion and exclusion criteria. The blood and saliva samples were collected in specified protocol in every subject. Glucose and Sialic acid levels were measured in serum and saliva samples along with serum HbA1C levels. The data was collected and analysed statistically. The age group of the study subject varied from 35 to 70 years. More than 55% of study subjects were <45 years in both the groups. The male and female ratio was approximately 60:40. The fasting blood sugar i.e. glucose (FBS) in type 2 DM ranged from 100 to 335 mg/dl (Mean: 155.8 mg/dl) where as in control group range was from 67 to 132 mg/dl (Mean: 88.9 mg/dl). Salivary glucose level in type 2 DM was from 10 to 30 mg/dl (Mean 20.4 mg/dl) but in the control ranged from 6.5 to 17.6 mg/dl (Mean: 9.35 mg/dl). The HbA1c level ranged from 7 to 10.8 (mean: 8.35) in type 2 DM and from 4.7 to 7.9 (mean 5.5) in control group. Mean +SD levels of sialic acid were comparatively high in both blood and saliva samples of cases than controls. The control group showed the values as 6.829 +0.162 in blood and 2.995 +0.070 in saliva. The cases had the values as 10.092 +3.45 in blood and 4.25 + 0.656 in saliva; significantly higher than the control group. Statistically strong correlation was observed between the sialic acid levels in blood and saliva. Further sialic acid levels also correlated strongly with the markers of Diabetes mellitus in blood and saliva. The levels of inflammatory marker sialic acid are found to be raised in Diabetes Mellitus in blood as well as in saliva. Sialic acid levels in blood and saliva of Type II Diabetes patients can be used to predict the risk of cardiovascular disease.

P023

Lipid Profile and Glycosylated Hemoglobin Status of Gestational Diabetic Patients and Healthy Pregnant Women

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Alteration in insulin sensitivity in Gestational diabetes mellitus (GDM) not only affects glucose homeostasis but also affects lipid metabolism as well. Glycosylated hemoglobin (HbA_{1c}) gives us an idea of blood glucose control prior to the actual estimation of blood glucose. The objective of the study was to determine serum lipid profile, blood glucose levels in oral glucose tolerance test (OGTT) and HbA_{1c}in GDM patients and healthy pregnant women. Thirty healthy pregnant women (controls) and thirty age and gestation matched women with singleton pregnancy; newly diagnosed with GDM satisfying the inclusion and exclusion criteria were included in the study. The serum was analysed for lipid profile, blood glucose and HbA_{1c}. The results showed that fasting blood glucose level, blood glucose level after 1 hour and 2 hours after 75 gm oral glucose administration (OGTT) were significantly higher in patients than in

controls ($p < 0.001$). HbA_{1c} was significantly higher in GDM patients as compared to controls ($p < 0.001$). There was a significant increase in serum cholesterol, serum triglyceride and serum VLDL level in cases when compared to healthy pregnant women ($p < 0.05$). Our study suggests that timely estimation of blood glucose, lipid profile and HbA_{1c} is very essential to improve adverse pregnancy outcomes in GDM as well as we can avoid further deterioration of the disease process by early detection and prompt treatment.

P024

Correlation of Serum Insulin and Procalcitonin with Insulin Resistance by HOMA in Type 2 Diabetic Patients

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Diabetes Mellitus is most common endocrinological disorder characterized by metabolic abnormalities and long- term complications. Also, Type 2 DM and Insulin Resistance leads to chronic low grade inflammation. Procalcitonin (PCT) is a new reliable marker of inflammation. The objectives of the study were to find correlation between serum Insulin and Insulin resistance by HOMA and to find role of Procalcitonin in Type 2 Diabetes. The study subjects included 100 newly diagnosed Type 2 Diabetic Patients having FBS > 126 mg%. All routine investigations were performed. Serum Insulin was estimated using ELISA. PCT was performed on i-chroma. Insulin resistance was calculated using following formula: - HOMA = Insulin (μU/L) X [Glucose (mmol/L)/22.5]. Patients were considered insulin resistant if HOMA ≥ 2.6. Statistical Analysis was performed using SPSS16 version. The Mean ± SD values of HOMA, Serum Insulin and PCT were 8.32 ± 3.79, 21.29 ± 8.64 and 3.88 ± 2.28 respectively. There was a positive significant correlation between these indices ($p < 0.001$). Out of the patients who had IR by HOMA, only 80% were detected having IR by Insulin levels. The present study suggested that Insulin levels were sensitive as well as specific as HOMA-IR in assessment of IR in diabetic population. Serum Insulin alone can also serve as predictor of Type 2 Diabetes. Also, there is a role of calcitonin related system in occurrence of DM.

P025

Association of C-Reactive Protein and Lipid Accumulation Product with Fasting Blood Glucose Levels in First Degree Relatives of Type-2 Diabetics

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Diabetes mellitus is characterized by chronic hyperglycemia and there is strong association of development of Type-2 diabetes mellitus with a family history. The first degree relatives of type-2 diabetics are more prone to develop diabetes in later life. CRP, a marker of systemic inflammation is associated with development of Type-2 diabetes mellitus, metabolic syndrome, and Coronary artery disease. LAP, an index of central lipid accumulation has also been associated with type-2 diabetes mellitus, metabolic syndrome and heart diseases. To find an association of CRP and LAP with fasting blood glucose levels in first degree relatives of type-2 diabetics. Fifty first degree relatives of type-2 diabetics were taken as subjects and 50 age and sex matched individuals without any history of diabetes mellitus in family served as controls. CRP was estimated using Nycocard reader. Fasting blood glucose, Fasting TGs estimations were done using fully auto analyser. Waist circumference values (in cm) were also taken and LAP score was calculated by the formula: (WC-58)*TG mmol/l in females and (WC-65)*TG mmol/l in males. The mean values of CRP, LAP, FBS in patient group were found to be 6.05 ± 1.5, 106.8 ± 40.7, 133.0 ± 18.9 respectively compared to controls 2.59 ± 1.2, 25.7 ± 14.6, 78.7 ± 8.2. A highly significant correlation of LAP and CRP ($p < 0.01$) was found in patient group. A significant correlation of CRP and LAP score with FBS ($p < 0.5$) was found. This study suggested that first degree relatives of type-2 diabetics are at increased risk of metabolic syndrome, diabetes and coronary artery disease.

P026

Duration of Diabetes as a Factor Influencing Oxidative Stress in Patients with Diabetic Macular Edema Post Anti-VegF Therapy

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Anti-VEGF therapy is the principal treatment for diabetic macular edema. Duration of the diabetes is a factor found to influence the pathogenesis of the diseases associated with diabetes. This study aimed at evaluating the response of patients, with different duration of diabetes, to anti-VEGF therapy, with respect to plasma oxidative balance. The objective was to evaluate the influence of duration of the diabetes on plasma levels of malondialdehyde [MDA], thiols and nitric oxide [NO] in patients with diabetic macular edema [DME] post anti-VEGF therapy. The study included 38 patients with DME, undergoing anti-VEGF therapy were included. Group I involved patients with diabetes duration ≤ 5 years; Group II, 5–10 years and Group III with ≥ 10 years. Blood samples were collected from all the patients before and a month after the anti-VEGF therapy. MDA, thiols and NO were estimated. Mean MDA levels was found to be increased in Group II [From 0.59 ± 0.10 to 0.75 ± 0.28 nmol/ml] which is unaffected in Group I. Thiol levels did not change among any of the groups. NO level was significantly raised in all the groups, maximum in Group III [from 76.67 ± 3.3 to 83.012 ± 4.15 μmol/L; $p < 0.05$]. Anti-VGF therapy has increased oxidative stress in DME. Patients with the duration of the diabetes >5 years are highly susceptible for the oxidative damage. Special care and regular follow up may be needed for these groups after the therapy, to overcome the side effects.

P027

Relation of Serum Uric Acid with the Components of Metabolic Syndrome

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Metabolic syndrome has become a worldwide epidemic. Serum uric acid (UA) level has been suggested to be associated with factors that contribute to the metabolic syndrome. However, the association between metabolic syndrome and UA has not been elucidated. The aim of this study is to see relationship between Serum uric acid (UA) level and the number of components that contribute to the metabolic syndrome, and which component was associated most with higher serum UA level. It was a cross sectional study compromising of 95 cases of metabolic syndrome which reported in tertiary care hospital in 3 months (May to July 2015). Data was collected by universal sampling method. Patients having any ≥ 3 of the 5 risk factor of metabolic syndrome were included and patients with renal disease were excluded. Analysis was done by using Spss16.0 version. Uric acid level was measured by primary investigator. The mean age of case was 59.07 ± 10.2 with male predominance 2:1. Metabolic syndrome distribution according to risk factors was 57, 31, and 7 respectively. Mean uric acid levels among male was 4.2 ± 2.3 and among female 4.1 ± 1.4. There was significant association between hyper triglyceridemia, diabetes, waist circumference with uric acid level ($p < 0.05$). Abnormal TG had the most influence on serum UA. A prospective study is warranted to determine if the prevention or treatment of hyperuricemia affects the development of metabolic syndrome.

P028

Association of Fetuin-A with Dyslipidemia in Young Individuals

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Introduction:
Fetuin-A is a multifunctional hepatic secretory protein that inhibits action of insulin. It is also predictor of cardiovascular mortality and is associated with dyslipidemia in non-diabetic coronary artery disease. The present study was conducted to evaluate the levels of Fetuin A in young individuals belonging to the age group of >18–35 years, and find the correlation if any between fetuin A and lipid profile. The present study was conducted in the Department of Biochemistry GMC Amritsar. The individuals were selected by conducting door to door survey of Amritsar and Tarn Taran district. Individuals belonging to the age group of >18–35 years were recruited for the present study. A total of 742 individuals gave their consent to join the study. Lipid profile complete along with Fetuin A was estimated. BMI and Non HDL cholesterol were calculated. The variations in all the parameters were studied according to tertiles of Fetuin A. It was observed that the levels of Triglycerides, Total Cholesterol, LDL, VLDL and Non HDL cholesterol were highest in the highest tertile of Fetuin A (all $p < 0.05$). In multivariate regression analysis Fetuin A was independently associated with triglycerides and Non HDL cholesterol. Fetuin A is positively associated with dyslipidemia, thus it may predict visceral adiposity and dyslipidemia thus placing the individuals with increased levels at an increased risk of CVD.

P029

Evaluation of Lab Tests in Patients on Treatment for Type 2 Diabetes Mellitus

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Worldwide increase in Diabetes is also reflected in India with Bangalore leading with one in eight people diagnosed as type 2 Diabetics. Lab tests have been extensively used in diagnosis, classification, prognosis and monitoring treatment. Higher tests like Insulin, C-peptide, microalbumin Hba1c are used for diagnosis and monitoring the diseases. C-peptide and insulin have common precursor but different half lives of 30 and 5 minutes respectively. C-peptide is not utilized so is a good tool for Insulin regulation. Urinary microalbumin is utilized to assess nephropathy while Hba1c is used to monitor long term glucose control. Together they reflect diseases progression. The objective of this study was to establish a correlation between the different markers in patients undergoing treatment for type 2 Diabetes and to establish significance of each in comparison to glucose levels. The study included 110 patients with known Type 2 Diabetes of both sexes. Samples collected as per protocol and tested for Glucose, Hba1c, Insulin, C-Peptide and microalbumin. Control group of 50 normal individuals of both sexes were also taken. All clinical details were taken from referring physicians. Statistical analysis was done. There is significant correlation between the various marker tests and the plasma glucose values as shown by the statistical analysis. The importance of each test was calculated by using the t test. There is significant correlation between plasma glucose and the other markers. Although more cost is involved clinicians should request more for these tests as they help in early intervention to prevent prolonged poor diabetic control.

P030

Study of High Sensitivity C-Reactive Protein in Type 2 Diabetic Nephropathy

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Type II Diabetes is now recognized as inflammatory condition associated with insulin resistance and abnormal endothelial vascular reactivity. Hs-CRP has long half-life, stability with no circadian variation, therefore is one of the best markers of vascular inflammation. There is also relation of inflammatory biomarkers and glomerular filtration rate in type II DM. Thus we planned this study. We sought to determine and compare serum high sensitivity C-reactive protein (hs-CRP) in type II diabetics with nephropathy and type II diabetics without nephropathy. Institutional ethics committee's permission was taken before study.50 patients in the age group of

18–65 years of type II diabetes with nephropathy (Group I) and 50 type II diabetic patients without nephropathy (Group II)were included in the study. Informed consent of the patient was taken. Fasting and postprandial blood glucose, serum hs-CRP, creatinine, urea, and urine albumin was assessed. The mean age group in Group I was 59 +11.48 S.D and Group II were 56.2 +9.28 S.D years. Hs-CRP levels in group I was 6.71 ± 3.4(S.D) mg/L which was higher and statistically significant ($P < 0.001$) as compared to group II 1.8 ± 0.5(S.D) mg/L. Also serum hs-CRP was significantly associated with blood glucose ($p = 0.001$), serum creatinine ($p = <0.001$) and urine albumin ($p = 0.01$). The increase in hs-CRP levels can be used as a marker and patients with high hs-CRP should be screened for nephropathy. Thus hs-CRP can be used as prognostic marker in patients with type II diabetes for progression to nephropathy.

P031

Spectrum of Liver Disease and Diabetes

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Diabetes is characterized by hyperglycemia and dyslipidemia caused by islet β-cells being unable to secrete adequate insulin in response to varying degrees of long-standing insulin resistance, which poses an enormous burden on modern societies owing to its worldwide explosion. Liver disease may cause or contribute to, be coincident with, or occur as a result of diabetes mellitus. The pathway leading from liver diseases to diabetes and back from the latter to the progressive liver disease is a vicious circle. The complex and bi-directional relationship linking the liver and diabetes has recently gained intense new interest. This article addresses the role of the liver in normal glucose homeostasis and discusses a variety of liver conditions associated with abnormal glucose homeostasis. This association may explain the pathogenesis of the liver disease or of the abnormal glucose homeostasis, or may be purely coincidental. Data support non-alcoholic fatty liver disease as a risk factor for the development of diabetes which is, in turn, a major contributor to progressive liver disease.

P032

Glycemic Index, Glycemic load and Risk of Type 2 Diabetes Mellitus in Indian People

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The calories coming from carbohydrates can be classified by their post-prandial glycemic effect, called the Glycemic Index or the glycemic load. The aim of study was an effort to know about GI concept in risk management for chronic disease such as diabetes and in controlling the blood glucose level. The study included 200 volunteer subjects of both sexes between the age group of 45–60 years with established type 2 Diabetes Mellitus. These subjects were divided into two groups. Group 1 ($N = 100$) was advised to consume low GI diets for 60 days while the Group 2 ($N = 100$) served as the control group by consuming diets as per their previous daily routine. In the following 60 days study the reverse scheme was followed by two groups. All subjects were examined for OGTT by GOD POD method and HbA_{1C} by Ion Exchange Resin Method at the start of the study (zero day) at 60 days and 120 days. There was no significant difference between the values of OGTT and HbA_{1C} for Group1 and 2 at start of the study (Zero day). At 60 days, the mean OGTT and HbA_{1C} values for group 2 were increased significantly when compared to that of group 1. At 120 days, values of mean OGTT and HbA_{1C} for group 1 were increased significantly when compared to that of group 2. Thus we conclude that as the prevalence of diabetes is rapidly rising all over the globe at an alarming rate, the GI of the foods commonly used in various states of India should be determined to help diabetic patients in selecting foods that would help in better managing their chronic condition.

P033

Correlation of Inflammatory Marker in Diabetes Associated with Liver Markers

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Diabetes observed in general population is an important risk factor for coronary heart disease. 12.6% of women and 9.3% of men are obese. Being overweight increases the likelihood of developing type 2 diabetes and cardiovascular disease (CVD). Understanding the pathogenesis and preventing long term complications have been major goals of research in diabetes mellitus (DM). Circulating levels of several inflammatory markers rise in individuals with long standing diabetes and increase risk of developing a chronic disease. In particular, elevation of plasma C-reactive protein (CRP), a nonspecific acute-phase reactant that is easily and reliably measured, has strong predictive power for cardiovascular events in diabetic patients. The aim of this study is to evaluate serum ALT, ALP, AST (markers of liver function), blood glucose and high sensitivity C reactive protein (hs-CRP) level (an inflammatory marker) in type 2 DM subjects, Further, we investigated correlation between these parameters. Fifty diabetic patients with elevated liver function parameters were included in this study.

Mean serum ALP and fasting blood glucose was 177.09 ± 65.43 mg/dl and 155.71 ± 14.84 respectively. Hs-CRP, ALP and AST are expressed in median with interquartile range and concentration was 104.8(19.2, 162.2), 65(41, 87) and 91(71,107) respectively. The concentrations were significantly higher ($p < 0.001$). Further significant positive correlation was observed between ALP and hs-CRP concentration as well as both with AST and ALT. We conclude that oxidative stress and inflammation appears to be a key component and also associated with poor glycemic control and further pathogenesis of diabetes and its complications.

P034

Paraoxanase and Apolipoprotein B in Hypertensive Patients

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Hypertension is the most important public health problem and is also quantitatively the most important risk factor for premature cardiovascular diseases, ischemic heart diseases globally. Hypertension alone is a major cause of atherosclerosis that leads to heart attacks and strokes. Apolipoprotein B (Apo B) is a component of chylomicrons and LDL-Cholesterol which are associated with the development of atherosclerosis. Human serum paraoxanase1 (PON1) is an enzyme that is bound to high-density lipoproteins (HDL). It plays a key role in protecting this lipoprotein and biological membrane against oxidative damage. PON1 is hypothesized to be an indicator of the risk of atherosclerosis and coronary artery disease development. Institutional Ethics Committee permission was obtained for carrying out this study. Total of 136 subjects were included in this study and after obtaining written consent, 4 ml of venous blood was collected and used for the estimation Of Apo B, PON1. Serum Apo B was measured by standard traceable assay, Serum PON1 measured spectrophotometry method. The exclusion Criteria were: alcoholic, smoker, pregnancy, any chronic disorder, liver and renal dysfunction. The inclusion criteria for cases: patients with history of HTN and for controls were: age matched healthy volunteers. The results showed that APO B ($p = 0.000$*) was significantly increased and serum PON1 activity ($p = 0.002$*) was significantly decreased in hypertensive patients when compared with that of controls respectively. Both Apo B and PON1 have a potential to be a prognostic parameters and may help in management of hypertension.

P035

Lipoprotein (A) and Risk of Type 2 Diabetes Mellitus

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Lipoprotein (a) - atherogenic and thrombogenic molecule has been associated with risk of cardiovascular disease but its role in type 2 diabetes is still unclear. The objective of the study was to find out if there is any association between lipoprotein (a) level with type 2 diabetes. The study included 200 type 2 diabetic subjects and 101 age and sex matched controls. Fasting blood samples were collected for analysis of lipoprotein (a). Results obtained were: 130 type 2 diabetic and 73 control subjects had less than 30 mg/dL of lipoprotein (a) with the mean of 14.41 ± 8.17 and 13.87 ± 7.79 respectively. 70 type 2 diabetic and 31 control subjects had more than 30 mg/dL of lipoprotein (a) with the mean of 59.81 ± 32.06 and 58.39 ± 21.04 respectively. There is no significant difference observed between the groups

P036

Study of Thyroid Profile in Type 2 Diabetes Mellitus - A Hospital Based Study

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Diabetes mellitus and thyroid dysfunction are two most common endocrinal disorders encountered in clinical practice with an interdependent relationship. DM appears to influence thyroid function in two sites, firstly at level of hypothalamic control of TSH release and secondly at the conversion of T4 to T3 in the peripheral tissues. Marked hyperglycemia causes reversible reduction of the activity and hepatic concentration of T4-5-deiodinase, low serum concentration of T3, elevated level of reverse T3 and low, normal or high level of T4. On the other hand untreated hyperthyroidism is associated with the reduced half-life of insulin, enhanced release of biologically inactive insulin precursors and reduced C-peptide to proinsulin ratio. The aim of the present study was to evaluate the prevalence of thyroid disorder in subjects with type 2 diabetics and the effect of type 2 diabetes mellitus on thyroid function. It was a cross sectional retrospective randomized hospital-based study of 100 Type 2 diabetic patients and 100 non diabetic subjects of $\geq$ 35 years of ages. The study subjects were investigated for Total Triidothyronine (T3), Total Thyroxin (T4), Thyroid Stimulating Hormone (TSH), Fasting Plasma Glucose (FPG) and Post Prandial Plasma Glucose. We found a higher prevalence of thyroid disorder (29%) among diabetic subjects against14% prevalence of thyroid disorder in healthy subjects. Mean fasting blood sugar (142.12 ± 39.06 with p value $= 0.0$) and mean serum TSH level (4.57 ± 5.80 with p value $= 0.0027$) were increased significantly whereas mean serum T4 level (8.56 ± 2.05 with p value <0.0001) was decreased significantly in diabetic subjects. We found a higher prevalence of thyroid disorder in type 2 diabetic subjects with subclinical hypothyroidism being more common with subclinical hypothyroidism being more common. So they should undergo screening for thyroid disorder to detect asymptomatic thyroid dysfunction and better management of diabetes.

P037

Relationship of Cystatin-C with Fasting Blood Glucose and Glycosylated Haemoglobin in Chronic Kidney Disease Patients with Type 2 Diabetes Mellitus

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Long standing diabetes mellitus and poor glycemic control have a preponderence for the development of nephropathy. Cystatin C, a novel marker, has been extensively used in research studies to evaluate its role in predicting renal function. This study aims to study the relationship between cystatin C with fasting blood glucose (FBG)

and glycosylated hemoglobin (HbA1c) in chronic kidney disease patients with type 2 diabetes mellitus (CKD-T2DM). The objective of the study was to compare and correlate cystatin C, creatinine, HbA1c and FBG in CKD-T2DM cases and controls. Forty CKD –T2DM cases and 40 healthy controls were selected. FBG, HbA1c, serum creatinine were analysed using standard methods. Serum cystatin C was estimated by Particle Enhanced Immuno turbidometric Assay. eGFR was calculated using Cystatin C based CKD-EPI formula. Comparisons between groups were done using Independent sample t test (parametric data) and Mann Whitney U test (non parametric data). Correlation studies were done using Karl Pearson's test. Mean duration of diabetes mellitus was 15.40 ± 9.03 years. Cystatin C along with creatinine, FBG and HbA1c were found to be significantly increased in cases when compared to controls ($p < 0.01$). Cystatin C had a significant positive correlation with FBG ($r = 0.791$), HbA1c ($r = 0.795$), creatinine ($r = 0.849$) and a significant negative correlation with eGFR ($r = -0.870$). The study showed that cystatin C has a positive relation with FBG and HbA1c. In patients with CKD-T2DM, cystatin C can be effectively used to monitor renal function.

P038

Thyroid Dysfunction in Type 2 Diabetes Mellitus: A Prospective Study

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Diabetes has emerged as pandemic health problem and its prevalence is increasing at an alarming rate. Diabetes mellitus (DM) is a worldwide major problem and despite advances in treatment, large number of patients present with complications due to poor glycemic control. Type 2 Diabetes Mellitus (T2DM) accounts for 90–95% of diabetes. India leads the world with largest number of diabetic subjects earning the term "diabetes capital of the world. One of the possible factors that contribute to poor glycemic control is thyroid dysfunction, which tends to occur concomitantly with DM. The present study was undertaken to find out the prevalence of thyroid dysfunction in patients with T2DM in Western U.P. Present research is a hospital based prospective study carried out in 150 subjects(100 T2DM cases and 50 control)aged 35–65 yrs, attending Endocrine Superspeciality CSSH, OPD, in Meerut. Fasting blood samples were collected for FPG, lipid profile, and hormonal analysis (T3, T4 and TSH). Prevalence of thyroid dysfunction was found 31% in T2DM study group, whereas FPG, total cholesterol, triglycerides, were significantly higher in T2DM study subjects compared with control while HDL-C was significantly lower in T2DM. The thyroid profile; T3 in T2DM case was significantly decreased 1.37 ± 0.49 nmol/L as compared to control 1.64 ± 0.67 nmol/L. T4 levels were decreased in case, 83.14 ± 18.29 nmol/L compared with control 86.9 ± 16.07 nmol/Lwas insignificant. The circulatory TSH levels were increased significantly in T2DM 4.14 ± 4.77 μIU/Compared with control, 2.55 ± 1.94μIU/L. The prevalence of thyroid dysfunction was found 31% in T2DM as compared to control. Diagnosis and treatment of thyroid dysfunction is important in management of T2DM patients.

P039

A Study of Essential Trace Elements and Oxidative Stress in Type 2 Diabetes Mellitus and their Relationship with Other Biochemical Parameters

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Type 2 Diabetes mellitus (T2DM) is the most common metabolic disorder associated with increased morbidity and mortality due to chronic complications. Poor glycemic control increases the rate of glycation of proteins, lipids and DNA forming Advanced Glycation End-products, which are prone to oxidation. The critical balance between oxidative and antioxidant mechanisms in health is affected in T2DM leading to chronic diabetic complications. An increase in the generation of free radicals in T2DM reduces the activity of superoxide dismutase(SOD), the main antioxidant in the cells and increases the peroxidation of polyunsaturated fatty acids in the cell membranes giving rise to malondialdehyde (MDA), the marker of oxidative stress. A number of essential trace elements like zinc (Zn), selenium (Se), copper (Cu), and manganese (Mn) act as cofactors for enzymes with antioxidant activity. Few studies have been conducted which show that T2DM is associated with a deficiency of essential trace elements but their relationship with the oxidative stress and pathogenesis of T2DM and its complications has not been established. Keeping in view the high prevalence of T2DM among the UAE population, the present study was carried out to correlate the levels of essential trace elements(Zn, Se, Cu&Mn) with the parameters of oxidative stress(SOD, MDA) in T2DM and identify their role in the pathogenesis of biochemical abnormalities (like albuminuria, dyslipidemia and elevated HbA1C levels). A cross sectional study was conducted on 160 T2DM patients and 44 healthy controls from both genders and different ethnic groups, in the age group of 30–60 years. The study was approved by the university research ethics committee. A validated, pre-tested, questionnaire was used to record the demographic details of the participants. All routine and specific biochemical tests were performed using validated and standardized procedures. The intra-and inter-assay CVs were within the permissible limits. The data was analysed by SPSS Program, version 21.0 using standard statistical procedures. P value < 0.05 was considered significant. The mean SOD level showed a significant decrease while the mean MDA showed a significant increase ($p < 0.01$) in T2DM patients when compared with the healthy controls. The mean Zn, Se and Mn levels were markedly decreased while mean Cu level was markedly increased (both $p < 0.001$). The mean SOD, Zn, Se and Mn levels showed a significant decrease while MDA level showed a significant increase with HbA1C levels. The increase in Cu level was insignificant when compared with HbA1C levels ($p > 0.05$). Albuminuria was associated with a significant decrease in SOD and a significant increase in MDA levels. The decrease in Zn, Se and Mn was insignificant when compared to albuminuria. However, Cu showed a significant increase in patients with albuminuria ($p < 0.001$) (The parameters of oxidative stress (SOD & MDA) and trace elements did not show any significant relation with lipid parameters. ($p > 0.05$). A significant decrease in the levels of essential trace elements and SOD and an increase in MDA levels and

their association with HbA1C levels and albuminuria suggest the role of trace elements and oxidative stress in the pathogenesis of T2DM and its complications. An early nutritional intervention may arrest the progression of disease and development of complications. This is the area of our future research and discussion.

P040

Study of Trace Element (Mg and Cu) in Type2 Diabetes Mellitus Patients

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Diabetes mellitus is a metabolic syndrome affecting carbohydrates, lipid and protein metabolism. It is heterogeneous disorder characterized by hyperglycemia due to impaired glucose utilization, resulting from defect in insulin secretion, insulin action. In diabetes mellitus seen alter level of some trace elements (mg and cu) has been reported. These trace elements might have specific role in the pathogenesis and progress of the diseases. The study was done in the department of biochemistry, Gandhi medical college and hospital, Bhopal. The present study included of 100 human subject, divided into two groups in which group 1 (healthy control) having 50 subject, group 2 (Type 2 Diabetes mellitus) having 50 patients, all patients were selected randomly without any bias for age, sex, occupation, socioeconomic status and duration of disease. Serum Mg estimated by Calmagite method and serum Cu estimated by Calorimetric method. Mg level was significantly reduced ($p < 0.01$) in serum sample of Type 2 Diabetes mellitus patients compare to healthy subject. Cu level was significantly higher ($p < 0.01$) in serum sample of Type 2 Diabetes mellitus patients compare to healthy subject. In our result we found altered level of some trace metal (Mg, Cu) in Type 2 Diabetes mellitus and it is helpful in diagnosis and screening of disease.

P041

Serum Electrolyte Profile in Diabetes Mellitus - a Retrospective Cross – Sectional Study

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Decompensated Diabetes Mellitus with impaired renal function, acid-base disorders are often associated with electrolyte disorders. Analysis of electrolytes is often advised without a true indication in Diabetes Mellitus. So our objective was to analyse serum electrolyte profile in Diabetes Mellitus to determine whether routine measurement of electrolytes can be safely avoided in Diabetes Mellitus. This was a retrospective study of hospital records of all Diabetes Mellitus cases who were advised kidney function test during the period January 2014 - August 2015. Medical records and laboratory results of 190 Diabetes Mellitus cases could be retrieved and retrospectively viewed for clinical diagnosis, result of Laboratory investigation i.e. Fasting Blood Sugar, serum Urea, Creatinine, Na^+, K^+, Cl^-, Ca^{2+}, $PO4^{-}$. One hundred ninety Diabetes Mellitus cases were divided into 3 groups based on their serum Creatinine levels i.e. Group-A($n = 98$) serum Creatinine <1.5 mg/dl, Group-B ($n = 74$) serum Creatinine 1.5–3 mg/dl and Group- C ($n = 18$) serum Creatinine >3 mg/dl. Out of 190 patients 114 had electrolyte disorder. Serum sodium levels were altered in 45 % of Group-A patients, more evident in Group-B (64%) and Group C (89%). Approximately 51% Diabetes Mellitus cases had deranged Serum K + levels in Group A. Hyperkalemia and Hypochloremia were common electrolyte disorders both in Group-B and Group C. Group- A had Hypercalcemia and hyperphosphatemia whereas Hypocalcemia and hypophosphatemia were more commonly registered in Group-B (40%, 37% respectively) and Group-C (68%, 61% respectively). So it is concluded that in Diabetes Mellitus electrolyte derangements occur even with normal renal function. Routine measurement of serum electrolyte could not be avoided in Diabetes Mellitus.

P042

Measurement of Neck Circumference as an Indicator of Abdominal Obesity

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Obesity is defined as excessive body fat accumulation. It is a major risk factor for a number of chronic diseases like diabetes, cardiovascular diseases, cerebrovascular diseases and cancer. In the assessment of central obesity, various techniques are used: waist circumference, waist/hip ratio, body mass index (BMI) and neck circumference (NC). Neck circumference is an index of upper body fat distribution and can be used as a simple screening measurement to identify obesity. Moreover the Framingam heart study has demonstrated NC as an independent predictor of visceral adiposity. Few studies have also shown that NC helps in predicting the metabolic abnormalities beyond the classical anthropometric indices like BMI, WC. Thus this study aims to determine the correlation of neck circumference with abdominal obesity. A total of 104 volunteers aged between 38 and 60 yrs attending the Master health check-up clinic of Sri Ramachandra Medical College & RI participated in this study (54 men, 50 women). The anthropometric indices like Height in m^2, weight in Kg, neck circumference in cm, waist circumference in cm were measured. Body mass index (BMI) was calculated with weight and height. Pearson correlation was employed to evaluate the association of neck circumference with indices of abdominal obesity namely WC and BMI. The mean Neck circumference among males were 39.1 ± 3.2 and females were 35 ± 2.04, the mean Waist Circumference for males were 97.9 ± 8.7 and females were 91.7 ± 11.9, the mean BMI for males are 26 ± 3.5 and females are 30.7 ± 2.9. In both the genders there were positive significant correlations between neck circumference, body weight (men, $r = 0.661$; women, $r = 0.702$; $p = < 0.001$), waist circumferences (men, $r = 0.787$; women $r = 0.466$; $p = < 0.001$) and BMI (men, $r = 0.701$; women, $r = 0.585$; $p = < 0.001$). A significant association was found between NC and obesity indexes. Thus this study indicates neck circumferences (NC), along with other obesity indexes can be used as a simple, reliable and quick method of assessment for obesity.

P043

Malabsorption Parameters in Type 2 Diabetic Patients

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Most of diabetic patients report significant gastrointestinal symptoms. Entire GI tract can be affected by diabetes. Proteins, carbohydrates, fats, and most fluids are absorbed in small intestine. Malabsorption may occur in type 2 diabetic patients. The present study was planned to measure various malabsorption parameters in type 2 diabetic patients. The study enrolled 175 patients and 175 age and sex matched healthy controls attending Endocrinology Clinic in PGI, Chandigarh. Orocecal transit time (OCTT), small intestinal bacterial overgrowth(SIBO) and lactose intolerance were measured using non-invasive lactulose, glucose and lactose breath tests respectively. Urinary D-xylose and Fecal fat were estimated using standard methods. Out of 175 diabetic patients, 87 were males while among 175 healthy subjects 88 were males. SIBO was observed in 14.8% type 2 diabetic patients and in 2.8% of controls. There was statistically significant increase ($P < 0.002$) in OCTT in type 2 diabetic patients compared with controls. It was observed to be more delayed ($p < 0.003$) in patients who had SIBO than in patients without SIBO. Lactose intolerance was observed in 60% diabetic patients and 42.8% controls. Urinary D-xylose levels were also lower in case of diabetic patients but no significant difference was found in 72 hours fecal fat excretion among diabetic patients and controls. Urinary D-xylose and lactose intolerance in SIBO positive type 2 diabetic patients was more severe as compared to SIBO negative diabetic patients. From this study we can conclude that delayed OCTT may have led to SIBO which may have instigated the process of malabsorption among type 2 diabetic patients.

P044

Study of Serum Vitamin D and Insulin Resistance in Obese Adolescents

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This study was designed to find if there is any relationship between serum 25-OH-D levels and insulin resistance in obese and non obese adolescents. Vitamin D deficiency in obese adolescent is strongly associated with increased risk for diabetes, hypertension and metabolic syndrome. Vitamin -D insufficiency is a risk factor for developing impaired glucose in childhood obesity is associated with insulin resistance in obese adolescents. In our study, we examined the relationship between vitamin-D and insulin resistance in obese adolescents. The study group included 50 obese adolescent aged (17–19 years) and compared with 50 non-obese controls were selected. Anthropometric data were collected and fasting plasma glucose was estimated by (GOD-POD) method, serum Insulin was estimated by (FEIA) method and insulin resistance was calculated by using (HOMA-IR) and serum (25-OH-D) was measured by using ELISA method. The vitamin-D levels in obese adolescents are slightly lower than the controls. The insulin levels in obese adolescents are slightly higher than controls. Insulin resistance was significantly higher in subjects with higher BMI. We found by correlation analysis that HOMA-IR was dependent on degree of obesity and independent of (25-OH-D) level. The study concludes that in obese adolescents insulin resistance was affected more from BMI than (25-OH-D) levels. Lower concentration of (25-OH-D) is also a risk factor for developing insulin resistance independent of adiposity.

P045

Abnormally High HbA1c in a 58 years Old Female Patient

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HbA1c, the Glycosylated hemoglobin is formed in two steps by the non enzymatic glycation of HbA. The first step is the formation of an unstable aldimine (labile A1c and pre A1c). Hemolobinopathies may interfere with analysis of glycated hemoglobin (GHb) with falsely increased or decreased values, depending on the particular method and the hemoglobinopathy. This study was performed to understand the cause of abnormally high HbA1c (33.4%) in a 58 years old female patient with normal fasting Glucose of 86 mg/dL HbA1c was separated by Cation – exchange High Performance Liquid Chromatography (HPLC) using Bio – Rad Variant II Turbo HbA1c kit 2.0. Separation and Identification of abnormal Hemoglobin was done by using Bio - Rad Variant II β-thalassemia short program using ion exchange HPLC. DNA study was done by National Institute of Immunohaematology, Mumbai. HbA1c = 33.4% (4.2 – 6.0), Glucose fasting = 86 mg/dL (74 – 99), Hemoglobin = 14.1 g/dL. Abnormal peak at P2 region of 44.5% was identified by β – Thal Program. DNA study gave results of Hb Singapore with α – globulin gene mutation: Cd 141 (CGT → CCT). Hemoglobin mutation alters the charge of Hb variant peak. The retention times of HbA1c and Hb Singapore are virtually identical; the elution peaks of these two Hemoglobin fall in the same window in the chromatogram, thus producing falsely increased HbA1c value. The HbA1c results should be carefully reported keeping in mind the interference of Hb Variants with HPLC method especially when results are not correlating with blood glucose values.

P046

Emerging Role of NAD(P)H: Quinone Oxidoreductase 1 (NQO1) in Patients with Diabetes Mellitus and Diabetic Nephropathy

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NAD(P)H: quinone oxidoreductase 1 (NQO1) is a crucial mediator of cellular defence against oxidative stress (OS) however, its ability to reduce OS in diabetes mellitus and its complications remains unexplored. To evaluate the NQO1 levels and its association with OS markers in Type 2 diabetic patients (T2DM) with and without diabetic nephropathy (DN). This study comprised of 600 participants divided into three groups of 200 each: T2DM, DN and healthy controls (HC). Plasma NQO1 was quantified using ELISA, glutathione-S-transferase (GST) activity, reduced glutathione (GSH) levels in blood, ferric reducing ability of plasma (FRAP) and malonaldialdehyde (MDA) were estimated spectrophotometrically. Two-way ANOVA followed by post-hoc Tukey's test were used to compare NQO1 activity and OS markers. Highest NQO1 activity was observed in diabetes mellitus followed by diabetic nephropathy and both were significantly higher ($p < 0.05$) as compared to HC. A similar trend was observed in GST activity. However, GSH and FRAP levels were found to be lowest whereas MDA levels were highest in DN as compared to DM and HC. In all patient groups NQO1 and GST showed a significant negative correlation with MDA, whereas a significant positive association was observed with GSH and FRAP ($p < 0.01$). Increased NQO1 activity observed in T2DM is probably induced as a compensatory mechanism in response to OS and may have a protective role. However, NQO1 activity subsequently decreases when these patients advance to DN, probably due to uremic toxins, leading to further augmentation of oxidative stress.

P047

Study of Lipid Profile Levels in Diabetics and Non-diabetics Taking Total Cholesterol/HDL Ratio and LDL/HDL Value in to Consideration

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The aim of our study was to evaluate changes in lipid profile levels in diabetic and non-diabetic males and females with special emphasis on role of Total cholesterol/HDL and LDL/HDL ratio in assessing the cardiovascular risk. A hospital based cross sectional study was conducted on 500 patients (250 males and 250 females) in the age group 25–80 yrs, were selected randomly for the study. Of the 250 males and females 125 were diabetic (FBS more than 110 mg/dl) and the remaining 125 were nondiabetic. All the participants underwent biochemical analysis of FBS, Lipid Profile (TC, TG and HDL). LDL and VLDL were calculated according to computational procedures of Freidwald. Biochemical and statistical analysis was done and all the selected patients and values were expressed as Mean ± SD ± SE. Camparisons of male and female diabetics were made with their non-diabetic counterparts. TC/HDL and LDL/HDL ratios were calculated to assess the cardiovascular risk. The mean value of FBS, TC, TG, LDL for diabetic males and females were higher than non-diabetics and HDL cholesterol values were found to be lower than non-diabetics. TC/HDL and LDL/HDL ratios were found to be much higher in diabetics than non-diabetic in both males and females. We conclude that dyslipidemia and diabetis mellitus go hand in hand.

P048

Serum Micronutrients, Lipid Profile and Their Inter Relationship in Patients with Type Ii Diabetes

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Diabetes is often associated with metabolic syndrome that is high blood pressure and abnormal lipid profile. Micronutrients mostly refer to vitamins and minerals. Micronutrients mainly exert their function as enzyme cofactors and transcription factors thus controlling the metabolic pathways. Metabolic syndrome is often characterized by various other clinical features like disturbance in glucose and insulin metabolism, obesity and abdominal fat distribution. **The objective was** to determine the difference in serum zinc, copper and magnesium and calcium levels in diabetic patients and to assess if there is a correlation between the micronutrients and metabolic parameters. The study was conducted in the Departments of Biochemistry and Medicine, Kasturba Medical College, Manipal. A total of 48 patients of both sex in the age group of 40- 75 were included in the study. 21 patients were included as controls and 27 patients were considered as cases. Serum zinc, copper, magnesium and calcium were estimated using quantitative colorimetric assay kits. Lipid profile was estimated using Cobas 6000 autoanalyzers. The results indicate that there is a statistically significant increase in serum zinc level in the diabetic group ($p = 0.003$) compared to controls. There is no statistically significant correlation between the minerals and metabolic parameters was obtained in either of the groups. There exists a positive correlation between serum calcium and total cholesterol and negative correlation of total cholesterol with serum copper and magnesium in the diabetic group. However the reverse was observed in control group. A similar pattern was observed on correlation of triglyceride levels with copper and magnesium levels. However serum zinc showed a negative correlation with triglyceride in controls.

P049

Evaluation of Serum 25-Hydroxyvitamin D in Type 2 Diabetes Mellitus

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Vitamin D plays a role in calcium metabolism. Vitamin D deficiency may be associated with a range of diseases, including cardiovascular disease and type 2 diabetes and has been associated with impaired insulin action, through direct effect of vitamin D on the β-cell function. The aim of this study was to evaluate serum 25 - Hydroxy vitamin D levels in diabetes mellitus type 2. This hospital-based study was conducted on 45 diagnosed Diabetes mellitus type II patients and 25 non-diabetic age and gender matched control. Blood

sample was collected and serum25-hydroxy vitamin D levels were evaluated by ELISA technique. The mean values of serum 25-hydroxyvitamin D levels in study group and control group were 16.31 ± 14.03 and 18.75 ± 18.63 respectively (Normal Value of vitamin D = 30–74 ng/ml). The decrease in Vitamin D levels in study group verses control group shows no statistically significant association (p value >0.05). In this study we observed no significant association of serum 25-hydroxy vitamin D with glucose metabolism in diabetes mellitus type 2 pateints. Our results are consistent with recent observations of smaller studies performed in Austria (Pilz S et al) demonstrating that patients with Diabetes Mellitus type II do not shows significant association with serum 25-hydroxyvitamin D levels.

P050

Altered Expression of Cysteine Proteases (Cathepsin L and Cathepsin B) in Human Dilated Cardiomyopathy

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Dilated Cardiomyopathy or DCM is one of the most common heart muscle disorder associated with high mortality. Cathepsin L (CTSL) and B (CTSB) are lysosomal cysteine proteases implicated in maintenance of cardiac architecture and function. Altered levels of these proteases are associated with adverse cardiac remodeling contributing to the pathogenesis of DCM. The aim of the study was to investigate the role of (if any) of Cathepsin L and B in acute *in vivo* rat model of doxorubicin-induced cardiomyopathy and in DCM patient samples. Total CTSL + B activity, CTSL and CTSB activity was assayed spectroflurometrically in rat myocardium tissue and in PBMC's of human DCM (N = 29) along with age matched controls (N = 29). Immunohistochemical analysis was used to assess the expression of these proteases in rat heart tissue– sections. The enzymatic activities of CTSL and CTSB were correlated with clinical echocardiographic parameters in human DCM. A significant increase in expression level of CTSL but not in CTSB was observed in doxorubicin treated group of rats. However, in human DCM we observed significant higher enzymatic activity of these proteases as compared to controls with a strong negative correlation with left ventricular ejection fraction (LVEF). ROC based analysis reveals good discriminatory power of Cathepsin L and B in human DCM. This study for the first time demonstrates elevation in the expression of Cysteine Cathepsins in PBMC's of DCM patients and a positive association between activities of these proteases with severity of disease. Thus measurement of the activity of these proteases levels may prove useful in management of DCM.

P051

An Association of Serum Vitamin D, IL-4 Level and VDR Gene Polymorphism in CAD With or Without T2DM

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Coronary artery disease (CAD) is a leading cause of death in developed countries and is rapidly assuming epidemic proportions in developing countries as well. It has been shown that lower vitamin D levels appear to predict an increased risk of CAD mortality in patients with Type 2 Diabetes mellitus (T2DM). Coronary atherogenesis leading to CAD is an immunological phenomenon caused by foam cells i.e. transformed macrophages at the lesion site. Apart from the traditional role of vita D in calcium homoestasis lot of recent experimental evidences are available on role of vita D levels, VDR gene polymorphism, and vitamin D binding protein gene polymorphism in immune reaction as immuno modulators and nowadays are being considered as risk factors in generating coronary atherogenesis leading to CAD particularly in association with T2DM. Recent studies also provide that IL-4 exerts proinflammatory effects on vascular endothelium and may play a critical role in developing coronary atherosclerosis. So we set our aims for this study to investigate the association of Vitamin D, VDR gene polymorphism and serum IL-4 levels in CAD with or without T2DM. The study involves two groups of patients suffering from CAD with T2DM (n = 40) and CAD without T2DM (n = 40) attended emergency or coronary care unit of Lok Nayak Hospital, New Delhi. A total of 6 ml of blood sample was collected for estimation of serum Vitamin D and IL-4 levels by chemiluminescence immunoassay method and VDR gene polymorphism (exon ll, rs 2228570) by PCR-RFLP using *Fok1* restriction enzyme. Other relevant routine blood biochemistry tests were done by Beckman coulter fully automated analyzer using commercially available kits. Serum Vitamin D levels were decreased in both groups of patients, more significantly decreased in the presence of T2DM in CAD patients. Serum IL-4 levels were significantly higher in CAD with T2DM group as compared to CAD without T2DM group. No association could be found between VDR gene polymorphism (*Fok1*) and risk of CAD in T2DM and non T2DM individuals. No significant correlation was found between vitamin D and IL-4 levels in the patients of both groups. No significant association was observed between low 25-hydroxy vitamin D levels with VDR genotypes (*Fok1*) in both groups of patients. The association between VDR Fok1 polymorphism, vitamin D and inflammatory markers needs to be further explored in diabetic CAD patients. A bigger study involving a much larger number of patients would help to generalize the results of this study.

P052

Role of Homocysteine in Stroke and Myocardial Infarction

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Homocysteine is a normal intermediate in methionine metabolism and it is also a sulfur containing amino acid. Homocysteine is receiving a lot of attention these days as a new risk factor for a variety of diseases including coronary heart diseases, cerebrovascular diseases and peripheral vascular diseases. The aim of the study was to estimate homocysteine level, lipid profile in myocardial and stroke disease patients and compare it with control subjects. In this study 30 myocardial infarction(MI) and 30 stroke patients admitted to medicine ward of Krishna hospital, Karad (Maharashtra), India were taken and these were compared with 30 same age sex matched healthy controls.5 ml blood sample was collected for estimation of homocysteine and lipid profile by using standard methods. Homocysteine (248.13%, 191.8%) was significantly increased in both MI and stroke patients as compared to controls. Triglycerides (TG) (27.08%, 7.18%) were significantly increased in MI patients while slight increase is seen in stroke patients. Ratio of total cholesterol/High density lipoprotein (TC/HDL) (27.3%, 27.08%), and ratio of low density lipoprotein/High density lipoprotein (LDL/HDL) (29.64%, 32.66%) is significantly increased in both MI and stroke patients. HDL (−16.8%, −17.1%) is significantly decreased in both MI and stroke patients as compared to controls. Very low density lipoprotein (VLDL) (25.64%, 8.46%). is significantly increased in MI and not significantly increased in stroke. TC (3.18%, 2.02%) and LDL (7.17%, 10.28%), were not significantly increased. Homocyseteine is very important biomarker of myocardial and stroke diseases and therefore it is very essential to consider the contribution of homocysteine as the principal biomarker in risk stratification for myocardial infarction which must be evaluated along with other risk factors.

P053

Association of Insertion/Deletion Polymorphism of Angiotensin-I Converting Enzyme Gene with type 2 Diabetes in North Indians

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Angiotensin-1-converting enzyme *(ACE)* gene has established substantial attention in the recent years as a candidate gene for hypertension and cardiovascular diseases. The present study was planned to investigate the association of *ACE* (I/D) polymorphism with high risk of type 2 diabetes (T2DM) in a North Indians. A total of 615 human subjects (315 T2DM patients and 300 controls) originated from a similar geographic location were included in this study. The mean age of diagnosis of T2DM was 50.1 ± 10.5 years. The mean BMI values did not differ significantly among diabetic and non-diabetic subjects (27.4 ± 4.6 vs 27.0 ± 4.7, p = 0.287). However, despite with non-obese BMI, patients had a pronounced abdominal adiposity reflected by their significantly higher waist circumference (37.1 ± 4.2 in patients vs. 35.2 ± 4.3 in controls, $p = 0.000$) and higher WHR (0.97 ± 0.07 in T2DM subjects vs 0.93 ± 0.06 in controls, $p = 0.000$). We observed pronounced central obesity in both patients and controls, even at the lowest BMI values (< 23 kg/m^2). Significantly, higher values of blood pressure (systolic and diastolic) were observed in diabetics compared to non-diabetic subjects. There was a significant difference observed in fasting glucose (183.5 ± 64.6 vs 96.8 ± 8.0, p = 0.00), HDL-C (38.9 ± 12.7 vs 41.5 ± 10.9, p = 0.001), HbA1c (8.7 ± 2.3 vs 6.8 ± 2.0, p = 0.00) and insulin (10.7 ± 6.7 vs 7.7 ± 3.3, p = 0.00) levels among diabetic and non-diabetic subjects. However, no significant differences between the diabetic patients and controls were observed in total cholesterol, triglycerides, LDL and Creatinine level. The genotyping data showed higher frequencies of DD genotypes in diabetic patients (34.6%) than that of control subjects (26.3%). The frequency of D allele was significantly higher in diabetic subjects (p = 0.004). Regression analysis of genotypic data showed a 1.9 folded increased risk of T2DM (OR = 1.9; 95% C.I. = 1.2–3.0; p = 0.005) in north Indian population. Similarly, individual D allele was associated with 1.4 fold higher risk of T2DM (OR = 1.4, 95% CI;1.1–1.7, p = 0.004). In conclusion, DD genotype of *ACE* gene may be associated with 1.9 folds increased risk of T2DM in north Indian population.

P054

Genetic Variants of ABO Blood Group and Coronary Artery Disease

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The *ABO* gene encodes for the blood group antigens which are expressed on the surface of RBCs, platelets, epithelium and the vascular endothelium. Several studies and recent GWASs have identified ABO as a locus for thrombosis, myocardial infarction, and multiple cardiovascular risk biomarkers. The objective of the study was to determine the association of genetic variants in the *ABO* genewith coronary artery disease (CAD). A total of 300 subjects including 150angiographically verified CAD patients' age and gender matched with 150angiographically verified controls were recruited for the study. Genotyping of variants A2 (deletion of C), B (G803C), O1 (deletion of G) and O2 (G802A) alleles, intronic variants rs8176746 (C/A) and rs8176722 (G/T) were performed by allele specific PCR and lipid profile was estimated for all the study subjects. Among the blood group alleles, the O1 (Cases-31%; controls-47%; p = 0.02) and the B allele (Cases-38%; Controls-16%; p = 0.001) were significantly associated with CAD while others (A1, A2 and O2 alleles) were non-significant. The variants of rs8176746 and rs8176722 were common in controls (23%;25% respectively) than in cases (10%;11% respectively) suggesting an atheroprotective role. The O blood group individuals had the highest mean HDL levels while in the B blood

group individuals the total cholesterol was higher in cases as compared to the controls ($p = 0.01$). This is an ongoing study and the preliminary results suggest the B blood group to be a risk marker for CAD while the O1 blood group to be atheroprotective.

P055

Oxidative Stress and Paraoxanase Status in Ischemic Stroke Patients

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Stroke is the leading cause of mortality worldwide. Oxidative stress is a characteristic of ischemic stroke. The event results in generation of free radicals leading to promotion of lipid peroxidation. Paraoxanase prevents oxidative modification of LDL. The aim of this study was to investigate the association between ox-LDL and Paraoxanase status in ischemic stroke patients by determining whether oxidative stress is an useful marker in ischemic stroke patients. The present study included 60 patients of ischemic stroke and 60 controls. All patients were in the age group of 55–85 years and admitted in ICU of base hospital attached to ACMS, Delhi. Plasma ox-LDL was estimated by ELISA method and serum Paraoxanase was measured by ELICO Spectrophotometer. Plasma levels of ox-LDL increased in stroke patients as compared to controls ($P < 0.001$) serum levels of Paraoxanase activity decreased in stroke patients as compared to controls ($P < 0.001$). Results indicate the presence of inflammatory response associated with stroke. We hypothesised that elevated ox-LDL levels and lower Paraoxanase activity may contribute for the development of oxidative stress. The present study was carried out to emphasize the importance of these markers for early diagnosis and therapeutic interventions in ischemic stroke patients.

P056

Serum Glutathione–S–Transferase and Total Antioxidant Capacity in Relation to Oxidative Stress in Coronary Artery Disease

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Oxidative stress has been implicated in the pathogenesis of Coronary artery disease (CAD). To combat oxidative stress, body has its own inherited antioxidant system consisting of various enzymes and molecules. Glutathione–s–transferase (GST) is an upcoming antioxidant enzyme having role in several diseased conditions. The aim of the present study was to evaluate the status of Glutathione –s –transferase in patients suffering from Coronary artery disease. The study was conducted in the department of Biotechnology, Swami Satyan and College of Management and Technology in collaboration with department of Biochemistry, Govt. Medical College Amritsar. 83 diagnosed cases of CAD were selected from the OPD and Medicine Ward of Fortis hospital, Amritsar. 100 age and sex matched apparently healthy individuals were taken as controls from the general population. Written informed consent was obtained from all the subjects. Serum lipid profile, Malondialdehyde (MDA), Reduced glutathione (GSH) and Glutathione-s –transferase were estimated with standard protocol. Total antioxidant capacity was also determined with commercially available kit. Triglyceride levels in CAD patients were significantly higher as compared to controls. CAD patients had significant low serum HDL cholesterol levels. Levels of other lipid parameters of both patients and controls were close to each other. Serum GST activity was significantly high and GSH level was low in CAD patients as compare to controls. Further more patients had high MDA levels as compared to healthy individuals. Total antioxidant capacity was observed to be relatively low in CAD patients as compare to controls. CAD patients were observed to have increased oxidative stress as shown by raised MDA levels. Low GSH level may be responsible for this increase in oxidative stress. Increased GST may be a mechanism to combat oxidative stress but it seems that it failed to compromise the level of MDA. Moreover total antioxidant capacity of the patients was low.

P057

Lipoprotein ‘A’ Gene Polymorphism and its Importance in Coronary Artery Disease

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Cardiovascular disease (CVD) is now the most common cause of death worldwide. CVD accounts for approximately 30% of deaths worldwide today. Studies indicate the prevalence of CHD to be between 7 and 13 per cent in urban and 2–7 per cent in rural India. There is compelling evidence from studies worldwide that Lipoprotein ‘a’ is a causal, genetic, independent risk factor for cardiovascular disease. Serum Lp ‘a’ levels may vary over a wide range and this is due to Single Nucleotide Polymorphisms and variable number of Kringle IV-2 domain repeats in the LPA gene located on chromosome 6q 26–27. Of the SNPs, few have been proved to increase serum Lp ‘a’ levels and in turn lead to increased risk of CAD beyond doubt. Our study is based on the intronic SNP rs1321196, which has been shown to be consistently associated with higher levels of Lipoprotein ‘a’ in various ethnic groups. The purpose of this study was to test the SNP rs1321196 and compare it with the serum levels of Lipoprotein ‘A’ in patients with Coronary Artery Disease. We tested 26 patients who were angiographically proven to have Triple Vessel Disease and compared them to 14 healthy age and gender matched control subjects. Mean Lp ‘A’ level in cases was 70.6 mg/dl and in control subjects were 50.9 mg/dl. The G → A polymorphism was found to be associated with increase in serum Lp ‘a’ levels (p value <0.044). The mean Lp ‘A’ levels were higher in cases when compared to control subjects. Homozygous Polymorphism increases serum Lp ‘A’ levels, whereas no direct correlation was observed between LPA polymorphism and disease. We require more number of subjects to consolidate our findings.

P058

Apolipoprotein A1 (G>A) Gene Polymorphism and its Correlation with Coronary Artery Disease in the Indian Punjabi Population

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ApoA1 is the major protein component of HDL and consists of 243 amino acids. Low serum ApoA1 levels are associated with increased risk of CAD. The ApoA1 gene is located on chromosome 11q23.3. The protective action of HDL and ApoA1 is attributed to their central role in reverse cholesterol transport (RCT). The present study was aimed to decipher whether the genetic variation in ApoA1gene is associated or not with CAD in the Indian Punjabi Population. Also to find the role of *ApoA1 G>A* polymorphism in delineating a subset population at higher or lower risk of CAD. A total of 300 CAD patients and 300 Normal individuals (controls) were analyzed. PCR-RFLP method was used to determine the DNA polymorphism in the ApoA1 gene, PCR products digested with restriction enzyme Msp1, followed by Agarose Gel Electrophoresis. Deviation from Hardy- Weinberg Equilibrium (HWE) was observed for this gene variant. The A- allele frequency was higher among Coronary Artery disease patients (53.8) compared to controls (45.5), $p = 0.004$, O.R = 1.38(1.11–1.75). Under recessive model analysis (*AA vs. GG + GA*) AA genotype of *ApoA1 G>A* polymorphism conferred $\sim$1 fold increased risk towards CAD susceptibility ($p = 0.002$, OR = 1.72(1.2–2.43). Overall, it may be concluded that A allele might be associated with increased risk towards CAD development in the Indian Punjabi Population.

P059

Cystatin C as a Potential Marker of Coronary Artery Disease

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Cystatin C, an established marker of renal dysfunction, is gaining importance in dysfunction of other organs (systems) as well. Preliminary studies indicated a role for cystatin C as a prognostic marker in coronary artery disease (CAD). The aim of the study was to assess the role of serum cystatin C levels in CAD and its spectrum. Study group comprised of 145 patients diagnosed as having CAD based on clinical and bio-chemical criteria. Control group included 66 age and sex matched subjects (non CAD cases) using the above mentioned criteria. In this study, significant increase of mean serum cystatin C levels was observed in CAD cases than controls. Highest mean cystatin C values were observed in Myocardial infarction (MI) than Unstable angina (UA) and Stable angina (SA). Highest mean serum cystatin C values were observed in CAD cases with risk factors, increased body mass index and waist circumference. Cystatin C plays an important role in the development of CAD and can be utilized aspotentialserum marker of CAD.

P060

Non-Fasting Samples for Estimation of Serum Lipid Levels in Patients with Coronary Artery Disease

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Evaluation of dyslipidaemia requiring fasting serum sample may not be possible in patients hospitalized in the acute phase. Evaluation of lipids in non-fasting sample was found to be useful but there are not many reports from India. One hundred and forty five patients with coronary artery disease (CAD) patients grouped as stable angina (SA), unstable angina (UA) and myocardial infarction (MI) and 66 healthy subjects were studied. Total serum cholesterol, triglycerides (TG) and high-density lipoprotein cholesterol (HDL) were estimated in a non-fasting serum sample. Increase in total serum cholesterol, TG, non-HDL along with a decrease in HDL levels was observed in CADpatients. The same trend was observed across the spectrum of CAD (SA, UA and MI). Non-fasting sample can also be utilized in order to evaluate CAD patients hospitalized in the acute-phase where waiting for obtaining a fasting sample may delay institution of specific treatment.

P061

Gold Nanoparticle Based DNA Sensor for Detection of Pathogen Causing Rheumatic Heart Disease

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Human rheumatic heart disease (RHD) is defined as impairment or permanent damage to the heart valves due to multiple attacks of acute rheumatic fever (ARF). ARF is a autoimmune response of infection with group A β-haemolytic *Streptococci.* RHD is the most significant abnormality of ARF caused by *Streptococcus pyogenes*. Biosensor can act as an efficient tool for early detection of RHD in human. The disease is initially started with throat infection and if it is not treated in time, it leads to RHD. The aim of the study was to develop nano-material based DNA sensor for quick, economical and early detection of RHD in human to prevent damage of mitral and aortic heart valves. Screen printed gold nanoparticle embedded carbon electrode, dendrimer, redox indicator, coupling agents, specific probe and buffer are required for electrochemical reactions. Carbon/gold nanoparticle/dendrimer/probe was fabricated for the early detection of *S. pyogenes.* The *sof* gene based biosensor was prepared by immobilization of 20 mer carboxyl labeled ssDNA probe on the composite electrode. The immobilized probe was hybridized with different concentrations of genomic DNA of *S. pyogenes* from throat swab samples. The electrochemical response was measured by cyclic voltammetry (CV) and differential pulse voltametry (DPV). The lower limit of detection of the sensor was found 1.2 pg from CV measurements. The sensitivity of the sensor was found to be 1299.20 $\mu A\ cm^{-2}\ ng^{-1}$. The total time for diagnosis of the disease takes only 30 min. Specific *sof* gene based probe was immobilized on gold nanoparticle/dendrimer to enhance the sensitivity of the sensor. The usual methods of detection of pathogen are either complicated or time consuming. Therefore, we have fabricated *sof* gene based sensor for

quick and early detection of pathogen to prevent damage of heart valves.

P062

Utility of Ischemia Modified Albumin and Cardiac Troponin I Measurements for Rapid Evaluation and Ruling Out of Stable Angina

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Myocardial ischemia is the main pathophysiological characteristic of Stable Angina. The manifestations of the myocardial ischemia are varied and multiple like chest pain, epigastric or arm discomfort, breathlessness, nausea and vomiting, however these symptoms may be subtle and are not recognized. Ischemia modified albumin is a form of human serum albumin in which the N-terminal amino acids have been modified by ischemia. The test has already been licensed by the US food and drug administration for diagnosis of suspected myocardial ischemia. The goal of present study was to assess diagnostic value of serum ischemia modified albumin and compare it with sensitive cardiac troponin I and Creatine Kinase-MB in stable angina. A diagnostic case control study was conducted on 60 patients of stable angina had taken from outpatients attending the cardiology department of hospital and 60 healthy age and sex matched volunteers formed the control group. Serum ischemia modified albumin level was estimated by albumin cobalt binding test using digital spectrophotometer, while troponin I was measured by immunofluroscence assay and creatine Kinase-MB was determined by immunoinhibition method. The sensitivity and specificity of ischemia modified albumin, troponin I and creatine kinase-MB for detection of Stable Angina were analyzed. The results of ischemia modified albumin, troponin I and creatine kinase-MB alone and in combination were correlated. Ischemia modified albumin, troponin I and creatine kinase-MB ($p < 0.01$) concentrations were significantly higher instable angina than healthy controls. Sensitivity and negative predictive value of CK-MB for detection of SA were 30.91% and 75.95% which were too less as compared to 87.27% and 93.52% respectively for the TnI and 84.55% and 92.98% for IMA. Ischemia modified albumin along with other gold standard cardiac biomarkers can improve early diagnosis of Stable Angina.

P063

Study of Oxidant and Antioxidant Status in Coronary Artery Disease

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Coronary artery disease (CAD) is a major cause of morbidity and mortality worldwide. Atherosclerosis, the main cause of CAD occurs as a result of constellation of various risk factors. Dyslipidemia, oxidative stress (OS) and inflammation are important risk factors for atherosclerosis. OS was also reported to influence response of CAD patients to treatment. Hence, identification of factors involved in the complex cascade of atherosclerotic process helps to predict risk of CVD and to devise effective therapeutic interventions. In this background, present study was taken up to study lipid profile, oxidant-antioxidant status and Myeloperoxidase (MPO) levels in patients with CAD and compare them with healthy controls.

Thirty patientsdiagnosedwith CAD and 30 controls were included after informed consent. Lipid profile [total cholesterol, triglycerides, HDL-cholesterol], OSmarkersmalondialdehyde (MDA), protein carbonyl content (PCO), MPO, and anti-oxidant marker, ferric reducing ability of plasma (FRAP) levels were measured in all subjects. LDL-cholesterol was calculated using Friedwald's formula. Statistical analyses were performed using SPSS version 11.5. Patients with CAD had higher triglyceride ($p = 0.012$) and lower HDL-cholesterol levels ($p = 0.000$) than controls. MDA, PCO and MPO levels were higher ($p = 0.000$) and FRAP levels were lower ($p = 0.000$) in CAD patients compared to controls. CAD patients were found to have OS as evidenced by elevated MDA, PCO, MPO and decreased FRAP levels. The increase in OS may increase their susceptibility to further oxidative tissue damage. Hence, therapeutic interventions targeted at reducing OS and improving anti-oxidant status through supplementation of anti-oxidants may help to improve the prognosis in these patients.

P064

A Study on the Association of Plasma Level of Magnesium, Copper and Zinc with Severity of Coronary Artery Disease

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Coronary artery disease remains the principal cause of death and disability in both developed and developing countries. Factors that cause endothelial dysfunction have received increased attention as a potential contributor for atherogenesis. Micronutrients play an important role in maintaining endothelial function as it act as cofactors for number of enzymes. Our aim was to determine plasma level of Copper, Zinc and Magnesium in CAD patients and its correlation with severity of coronary artery disease. Study group consisted of angiographically positive CAD patients ($n = 52$) and age and sex matched controls ($n = 50$). Severity of the CAD was assessed based on the number, location and percentage of stenosis of coronary vessels involved and SYNTAX score was calculated. CAD patients were classified into Grade I, II and III based on SYNTAX score. Level of Copper, Zinc and Magnesium in plasma and hemolysate was analyzed using atomic absorption spectrometry. Plasma Nitric oxide level was also analyzed. In our study, Plasma Copper level was found to be significantly high and Zinc copper ratio, hemolysate Mg level were found to be significantly low in CAD patients. The latter parameters were found to have a significant negative correlation with severity of the diseases and with the level of Nitric oxide. Even though a number of confounding factors can influence the Copper, Zinc and Magnesium levels in the body, our study shows that levels of these elements have a significant association with severity of coronary artery diseases.

P065

Loop Sided Redox in Buerger's Disease: A Possible Etiological Factor

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Free radical and antioxidants have undergone three clear cut temporal phases; first free radical acts as a "Pandora box of evil" (involved in >100 disease) and antioxidant as a miracle molecule especially nutrient antioxidant, second free radical as essential molecules in physiology (differentiation, apoptosis etc) and oxidative stress. Third re-emerging evidence of involvement of free radical at molecular level in genesis of the disease. Smoke releases many toxin in body and is the predisposing factor for Buerger's disease. Herein we argue that oxidative stress in smoke is a cofactor in the etiopathogenesis of Buerger's disease and only possible consequences in COPD. In this study 94 subjects (Healthy individuals- 68, Buerger's Disease-7 and COPD-19) were examined for GSH, SOD, CAT, GPx (hemolysate), peroxide level (TBARS), beta carotene, retinol, alpha-tocopherol and ascorbic acid in plasma. Relevant statistical exercises were done. All Buerger's patients were chronic bidi smokers (> 40/day). COPD patients had significantly raised oxidative stress (TBARS- 6.1 ± 3.3 nmol/ ml) then normal subject, only alpha tocopherol and GSH were lower then normal subjects. However in Buerger's patients OS was alarmingly high (TBARS- 10.1 ± 2.5 nmol/ ml) and nutrients antioxidants were low, both GPx and catalase activities were raised thereby suggesting increased production of H_2O_2. All data together suggests adversely titled redox sensitive events. We conclude that provoked oxidative stress as consequence in COPD but a possible etiologic factor in Buerger's disease.

P066

Prevalence of Thyroid Disorders in Clinically Suspected Population of Delhi by using Radioimmunoassay (RIA) and Immunoradiometric Assay (IRMA)

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The aim of this study was to determine the prevalence of thyroid disorders (Hypothyroidism and Hyperthyroidism) in clinically suspected population of Delhi by using Radioimmunoassay (RIA) and Immunoradiomatric Assay (IRMA) and to assess its significance. Fasting blood samples of 5350 patients (4280 female and 1090 male) upto 80 yrs of age attending various physician clinic all over Delhi were collected in BD vacutainer from Jan 2013 Dec 2014. Serum was separated and stored at 2–8°C till analysis. The samples were analyzed manually in batches of 50–100 samples on the same day of collection to avoid any deterioration of serum. Keeping in view the cost factor, only clinically suspected cases of thyroids disorders having signs and symptoms of hypothyroidism or hyperthyroidism were included in the study. Already known cases of any type of chronic illness or thyroid disorders were excluded. Free Triiodothyronine (FT3)/Free tetra-iodothyronine (FFT4) and thyroid stimulating hormone (TSH) were estimated by using Radioimmunoassay (RIA) and Immunoradiometric Assay (IRMA) respectively. PC-RIA, MAS, stratec, Germany, 12 well Gamma counter was used for measurements and calibrations. The results were analyzed age wise as well as gender wise and divided into four groups viz group-I, 0–20 yrs, group-II, 21–40 yrs, group-III, 41–60 yrs and group-IV, 61–80 yrs. The analysis of results was done within the same group and between different groups with respect to hypothyroidism and hyperthyroidism, subclinical hypothyroidism and gender. In the present study we have found an overall prevalence of thyroid disorders to be 27.29 % (1460, 22.06 % female and 1180, 5.23% male with female to male ratio of 4.31:1). The percentage of hypothyroidism, hyperthyroidism and subclinical hypothyroidism was 11.21 (8.95% female and 2.26 % male), 4.48 (3.88% female and 0.6% male) and 11.58 (9.21% female and 2.37% male) respectively. In the individual age groups viz group I-0–20 yrs, group-II-21–40 yrs, group-III-41–60 yrs & group-IV- 61 – 80 yrs, the overall prevalence of thyroid disorders were 2.8 %, 15.3 %, 7.66 % and 1.49 % respectively. Age group II made the highest proportion (3330, 62.24%) and also showed the highest prevalence of thyroid disorder (15.3%) with hypothyroidism more (12.0%) prevalent than hyperthyroidism (3.9%). The study showed theat relative percentage of thyroid disorder was approximately four times higher in female than male in all age groups except group l.

P067

Association of Hypothyroidism in the Pathogenesis of Polycystic Ovarian Syndrome (PCOS)

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To test the association of hypothyroidism in the pathogenesis of endocrine disarrangement in Polycystic Ovarian Syndrome (PCOS). 85 Polycystic Ovary Syndrome women in reproductive age attending the outdoor of North Eastern Indira Gandhi Regional Institute of Health & Medical Sciences, Shillong, Meghalaya from May 2011 to January 2013 were recruited to the study. PCOS was diagnosed by ultrasonographic finding of smooth sclerotic ovary with a thickened capsule & multiple follicular cyst or Elevated LH/FSH ratio with following clinical findings of oligomenorrhoea or amenorrhoea & acne or hirsutism. LH/FSH ratio greater than 2 was accepted as abnormal. Elevated LH/FSH ratio (LH/FSH > 2) was found in 70.58 % of the studied PCOS women (60 out of 85) whereas gonadotrophin ration were normal in 29.41% studied cases (25 out of total 85). Statistically significant differences were noted between groups with normal and elevated LH/FSH ratio in the following parameters: serum TSH, serum Prolactin, and serum Insulin levels. How-ever, there was no statistical difference noted in total Prolactin & insulin concen-trations noted between the study groups. The study showed LH/FSH ratio is not a characteristic attribute of all PCOS women as nearly 30 % of the individuals did not showed elevated LH/FSH ratio. Patients with Hypothyroidism constitute probably a subpopulation with increased adrenal androgenic activity leading to PCOS.

P068

Role of Calcium Markers in Detection of Bone Turnover

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Bone tissue undergoes remodeling via bone reabsorption and formation. In young balance is maintained but disturbed while aging causing bone loss and ultimately a high risk of bone fractures. Age-related bone loss is accelerated by impaired calcium intake and low levels of Vitamin D. Decreased precursors in skin and kidneys lower calcium absorption leading to PTH increase and bone reabsorption. To establish correlation between serum calcium marker levels and disease condition by analyzing data gathered for 18 months. Serum PTH, Vit D, Calcium, Phosphorus and Creatinine were evaluated for detection and utility in treatment monitoring. 170 cases were studied. These were compared to a normal population of 110. The Clinical data was obtained from the referring clinician. Standard sample collection procedures were used. PTH and Vit D, ECLIA and Calcium, phosphorous and Creatinine were analyzed. Data was subjected to statistical analysis. Statistical analysis showed good correlation between the disease state and the markers and also there was significant difference between the age groups and the genders. The calcium markers were higher in patient group than in the age matched control population. There was significant correlation between the calcium markers and the bone turnover as shown by the student's t test. Significantly lower calcium and Vit D values were found in individuals with higher PTH which also correlated well with Creatinine values in patients with kidney impairment. These markers are useful in assessing bone turnover and help in early detection and treatment follow up.

P069

Correlation of Thyroid Dysfunction in Primary Infertility and Menorrhagia

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Thyroid dysfunction is associated with a broad spectrum of reproductive disorders including infertility and menstrual irregularities. This study was done to determine the relationship between different degrees of thyroid dysfunction with infertility and menstrual irregularities.

Serum TSH, fT4 and fT3levels were analysed in 550 patients of primary infertility, 500 patients of menorrhagia and 500 controls using fully automated chemiluminescent immunoassay analyser. Hypothyroidism was further divided into subclinical hypothyroid (TSH- 6.0 to 9.9 μIU/ml) and overt hypothyroid (TSH $\geq$ 10 μIU/ml). In primary infertile women, 77.77% were euthyroid, 21.5% were hypothyroid and 0.7% were hyperthyroid. The prevalence of hypothyroidism in primary infertile women was comparable with that of controls(21.49% vs 17.66%). However, among the hypothyroid group, prevalence of overt hypothyroidism was more in the infertile women(70.7%) as compared to the controls(18.9%). In menorrhagia, prevalence of euthyroidism, hypothyroidism and hyperthyroidism was 77.77%, 21.5% and 0.7% respectively. There were significant differences in the levels of TSH, fT3 and fT4 between the menorrhagic women and control population ($p < 0.01$). The prevalence of menorrhagia was not much different between subclinical and overt hypothyroid females (43.5% vs 56.5%) in patient group. Hypothyroidism per se in not the cause of infertility. But, severe dysfunction of the thyroid may result in infertility. Thyroid function is significantly altered in menorrhagia. Thus, due to potential implications of thyroid dysfunction on ovulatory function and menstrual disturbances, screening is certainly indicated.

P070

Serum Prolactin in Subclinical and Overt Hypothyroidism

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Hyperprolactinemia is a common condition that can result from a number of causes including hypothyroidism. Prolactin secretion is controlled by prolactin inhibitor factor that is secreted from hypothalamus, factors like vasoactive inhibitory peptide (VIP) and thyroid releasing hormone (TRH) lead to increase in prolactin secretion. Objective of the study was to find out serum levels of prolactin and thyroid hormones in euthyroid, subclinical and overt hypothyroid cases. Consecutive patients presenting to endocrinology clinic of C S S Hospital, Meerut, India, for various thyroid related problems were segregated into two groups subclinical and overt hypothyroidism according to their diagnosis based on clinical examination and history, laboratory reports, inclusion and exclusion criteria. Newly diagnosed seventy five patients in each group were finally enrolled. Similar number of age and sex matched controls were selected. All subjects filled a predesigned questionnaire for the evaluation of hypothyroid symptoms. Thyroid profile for T3, T4 (total and free), TSH and prolactin were determined in all the subjects and analyzed. Prolactin elevation was found in 16 patients (21.33 %) with overt hypothyroidism and in 6 patients (8%) with subclinical hypothyroidism. The control group and subclinical hypothyroid patients exhibited no significant difference in terms of total and free T_3, total and free T_4. For TSH and prolactin on the other hand, a statistically significant elevation was found in patients with overt hypothyroidism when compared with subclinical hypothyroidism; and in patients with subclinical hypothyroidism when compared to the controls. A significant statistical difference was observed between the two groups of hypothyroid patients for all hypothyroid symptoms except alopecia and hirsuitism. The incidence of hyperprolactinemia in hypothyroidism was found to be higher when compared with normal controls. Thyroid function tests should be performed on patients with hyperprolactinaemia before performing further tests.

P071

Significance of Prolactin and TSH Levels in Infertile Females in Solan Region of Himachal Pradesh

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Fertility in female is maintained by hormonal milieu delicately balanced by hypothalamic-pituitary-thyroid-adrenogonadal axis. Hyperprolactinemia is a common problem in reproductive dysfunction affecting about one third of infertile women. Infertility is common accompaniment of disorders of thyroid functions. The study was designed to evaluate the role of prolactin in female infertility and to find its correlation with serum TSH levels. 50 patients with infertility (both primary and secondary) constituted the study group and 50 age- matched fertile females were categorized in the control group. The association between thyroid dysfunction and levels of serum prolactin was evaluated. Out of 50 patients, 33(66%) had primary infertility and 17 (34%) had secondary infertility. Serum prolactin in the study group (21.69 ± 18.97 ng/ml) was found to be high as compared to the control group (10.22 ± 5.40 ng/ml). Hyperprolactinemia was depicted in 26% infertile females. Hypothyroidism was found in 20% of infertile females with mean serum TSH levels to be significantly raised in the infertile group (3.88 ± 3.24 μIU/ml) as compared to control group (2.05 ± 1.07μIU/ml). There is a high incidence of hyperprolactinemia in infertile females as well as a greater propensity of thyroid disorders that emphasize the importance of screening of prolactin and TSH levels in these females.

P072

Prevalence of Thyroid Autoimmunity and Vitamin B_{12} Insufficiency in Patients with Thyroid Disorders

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Endocrine disorders are on the rise world over. Diseases of thyroid gland are among the most prevalent endocrine disorders in the world, second only to diabetes mellitus. Thyroid diseases are more prevalent in the sub Himalayan region even in post salt iodination phase. To assess the prevalence of thyroid autoimmunity and vitamin B_{12} insufficiency in patients with thyroid disorders at a tertiary care center of Himachal Pradesh. Thyroid function tests (T_3, T_4, TSH) along with anti thyroperoxidase antibody (ATA) and vitamin B_{12} levels were done by chemiluminescent immunometric immunoassay on Immulite 1000 system, after serum separation of 63 subjects. Study population comprised of patients above 18 years of age, who were advised thyroid function tests by the clinicians on an outpatient basis. Patients who were critically ill were excluded from the study. ATA level >50uIU/ml was taken as positive and Vitamin B_{12} <200 pg/ml were taken as insufficient (as per the previous study in the same region). Mean age of study population was 42.48 (± 12.32) years. 55.6% of the study population was found to be hypothyroid. ATA positivity was reported in 65% (41/63) of the samples. 63% (26/41) were found to be Vitamin B_{12} deficient among ATA positives. There was a positive association between thyroid autoimmunity and B_{12} deficiency (OR = 1.2). Intervention studies may be carried out to assess the role of insufficiency of Vitamin B_{12} in development of auto immune thyroid disorders.

P073

Elevated Iron, hs C - reactive protein and Reduced Adiponectin Levels in Benign Prostatic Hyperplasia

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Benign Prostatic hyperplasia (BPH) is a multifactorial disease. Elevated iron and inflammation are known to increase the growth of neoplastic cells and play a role in the development of tumors. The present study was designed to assess the levels of iron, hs CRP and adiponectin and their association with prostate size in BPH patients. 40 BPH cases and 40 controls were enrolled in the study. Iron, hs CRP and adiponectin were estimated in both the groups. Iron and hs CRP were significantly increased and adiponectin was significantly reduced in BPH cases when compared with controls. Iron ($r = 0.397$, $p = 0.015$) and hs CRP ($r = 0.341$, $p = 0.039$) were significantly associated with prostate size in BPH cases. Stepwise regression analysis showed that iron act as predictor of prostate size in BPH ($R^2 = 0.344$, $\beta = 0.576$, $p = 0.001$). We conclude that iron and hs CRP are elevated in BPH cases and associated with severity of BPH. Adiponectin was reduced in BPH cases, but not associated with severity of the disease.

P074

To Study the Association of Sp1 COLIA1 Gene Polymorphism with Bone Mass in Women of Sikkim

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Primary Osteoporosis in women is considered mainly due to the attainment of menopause but genetic factors also contribute to the pathogenesis according to many studies. Very few studies have been done to find the contribution of genetic factors to the pathogenesis of osteoporosis in women in India. This study was planning to analyze the Sp1 COLIA1 gene polymorphism and it relationship with bone density. A total of 75 patient with primary Osteopenia and Osteoporosis and 75 healthy control of age range 35- 65 years were enrolled in the study. The COLIA1 genotypes [SS, Ss & ss] were assessed by restriction enzyme digestion [Msc I] of DNA amplified by polymerase chain reaction. The frequency of genotypes in cases was [97.3% SS, 2.7% Ss and 0% ss] and in control was [100% SS, 0% Ss and 0% ss]. No significant association was found between COLIA1 genotypes and low bone density (Chi square = 2.027, df = 1, P = 0.155, RR = 0.493). The study suggests that the Sp1 COLIA1 gene polymorphism is very rare and is not indicative of genetic contribution to the pathogenesis of osteoporosis in women of Sikkim.

P075

Study of Thyroperoxidase Antibodies in Hypothyroid Pregnant Women of Lucknow and its Surrounding Areas

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Pregnancy results a series of profound physiologic changes that have a significant effect on maternal thyroid function. Both overt and subclinical maternal thyroid hormone deficiencies are common in pregnancy, and are associated with several adverse outcomes. Worldwide the most common cause of hypothyroidism in pregnant women is iodine deficiency. In the areas that are iodine sufficient the most common causes are autoimmune thyroiditis. This study was done to find out prevalence of TPO antibody in the hypothyroid pregnant women of Lucknow and its surrounding areas. The study was conducted at Era's Lucknow Medical College, Lucknow. 1000 pregnant women, coming for the first visit at Obstetric OPD, were screened for hypothyroidism. TSH was done using ELISA. Women positive for hypothyroidism were further screened for TPO antibody. The total prevalence of hypothyroidism was found to be 34.6%. Prevalence of hypothyroidism among individual trimesters was 38.21%, 34.16% and 30.39% in 1st, 2nd and 3rd trimesters respectively. Although apparent decreasing trend is seen in the prevalences, no significance difference was found. 14.1% of hypothyroid women were positive for TPO antibody and did not show any relation with the trimester and age in which the patient presented. High prevalence of hypothyroidism and TPO antibody positivity is seen among pregnant women of Lucknow and its surrounding areas although no significant association is seen between the two parameters.

P076

Study of Insulin Resistance and Lipid Profile in Hypothyroid Patients

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The effect of thyroid status on insulin sensitivity is of great interest but despite various studies there is conflicting data on this subject. Hypothyroidism has been associated with disorders of glucose and insulin metabolism involving defective insulin secretion in response to glucose, hyperinsulinemia, altered peripheral glucose disposal and insulin resistance. Thyroid dysfunction leads to alterations in glucose and lipid metabolism which is an important risk factor for cardiovascular diseases. The dyslipidemia and insulin resistance should be managed aggressively to reduce the impending risk. The study is aimed to evaluate insulin resistance and lipid profile in hypothyroid patients. A cross-sectional study in which 50 clinically diagnosed cases of hypothyroidism attending endocrinology and medicine OPD at SRMMCH&RC and equal number of healthy controls are selected for the study. Fasting blood samples are obtained for Glucose, TSH, FT_3, FT_4, Insulin & lipid profile. Glucose estimated by GOD-POD method, lipid profile by enzymatic method in auto analyzer, Thyroid hormones and Insulin estimated in TOSOH by Immunofluorometric method. Students't' test (SPSS version 21.0) and Pearson's correlation determines the association between insulin and lipid levels in hypothyroid patients. Altered insulin levels and lipid profile in hypothyroid patients when compared to controls indicating that these patients are at risk of cardiovascular disorders.

P077

Changes of Serum Lipids and Oxidative Stress Levels in Patients with Hypothyroidism

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Hypothyroidism is defined as failure of thyroid gland to produce sufficient thyroid hormone to meet the metabolic demands of the body. Untreated hypothyroidism can contribute to hypertension, dyslipidemia, oxidative stress, infertility, cognitive impairment and neuromuscular dysfunction. The aim of present study was to assess the association of hypothyroidism with lipid abnormalities and oxidative stress. The present study was carried out in Department of Biochemistry, Sri Aurobindo Institute of Medical Science and P.G. Institute, Indore. The study group comprised of 60 diagnosed hypothyroid patients between the age group of 21 – 70 years. Subjects were divided into two groups, 1st group was healthy control (n = 60), 2nd group was hypothyroid patients (n = 60). The following investigations were carried out-1) Estimation of lipid profile by standard commercial method, 2) Estimation of SOD by Marklund and Marklund method, 3) Estimation of plasma MDA by Jean C.D. *et.al* method. In our study serum total cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides and MDA levels were found significantly increased in hypothyroid cases compared to healthy controls with p value <0.001 whereas HDL cholesterol and SOD levels were found significantly decreased in hypothyroid cases compared to healthy controls with p value <0.001. Results of our study suggest that dyslipidemia and oxidative stress are associated with hypothyroidism.

P078

To Study Prevalence of Infertility in Patients of Hypothyroidism

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Infertility is defined as the inability to conceive after one year of regular intercourse without contraception. Currently the estimated rate of infertility is between 12 and 14%. Thyroid dysfunction is also a known cause to reduce the chances of pregnancy and also affect adversely the pregnancy outcome. Keeping in view the role of thyroid

hormones in normal pregnancy the present study was planned to access the prevalence of hypothyroidism (overt and subclinical) in infertile patients and find a correlation between thyroid and fertility hormones in these individuals. The present study was conducted in the Department of Biochemistry in association with department of Obstetrics and Gynecology, GMC, Amritsar. A total of 75 infertile females in age range of 20–35 years were included in the present study with an equal number of age matched normal females to serve as control. 49.3% of infertile females belonged to the age group of >19–25 years with duration of marriage upto 2 years, all these females were subdivided as normal, subclinical hypothyroidism and clinical hypothyroidism depending on the levels of TSH. A significant increase in levels of prolactin were observed in case of subclinical hypothyroidism and clinical hypothyroidism as compared to normal females (32.31 ± 23.06 and 55.05 ± 38.41 v/s 13.70 ± 8.31 ng/ml). TSH had a positive significant correlation with prolactin and LH whereas FSH levels were negatively correlated, both in subclinical hypothyroidism and clinical hypothyroid patients. Even subtle increases or decrease in the TSH levels may be clinical or subclinical can affect the fertility profile leading to infertility.

P079

Standardization and Validation of Simultaneous Immunoassay for Two Analytes Related to Thyroid Disorders

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Thyroid stimulating hormone (TSH) and thyroglobulin (Tg) form an important group of analytes for monitoring thyroid cancer patients post thyroidectomy. TSH and Tg are presently assayed by any of the conventional isotopic or non-isotopic assays. Simultaneous analysis of the required analytes for a disease can save assay cost, analysis time and sample volume. Multianalyte immunoassays (MAIA) requires an "antibody-chip", which is a suitable solid support containing required antibodies immobilized as small spots of ~1 mm diameter. The objective was to standardize and validate a MAIA for estimation of TSH and Tg. Non-competitive immunoassays were performed by immobilizing 0.5 µl (1 mg/ml) of monoclonal anti-TSH and polyclonal anti-Tg antibodies on highly micro-porous polycarbonate (PC) track-etched membranes (TEM). Mixture of ^{125}I labeled monoclonal antibodies against both analytes was used for detection. Mean spot intensity was determined by imaging with a PhosphorImager and analyzing the spots using ImageJ analysis software. Concentration of the tracer and time of incubation was optimized and the assay was validated using relevant parameters: cross-reactivity, sensitivity and range, precision and comparison with IRMA. The assays developed were sensitive (0.03µIU/ml for TSH and 0.1 ng/ml for Tg) with clinically useful working range (0.03–50µIU/ml for TSH and 0.1–250 ng/ml for Tg) using optimized concentration of tracer (100,000 cpm/100 µl of each analyte). The assay was precise and showed a good correlation with routine IRMA assays carried out in our laboratory, ($r = 0.98$, $p < 0.001$ for TSH and $r = 0.91$, $p < 0.001$ for Tg, $n = 41$). These results describe development of a sensitive, specific and cost-effective MAIA as an alternative to conventional immunoassays.

P080

Determination of Altered T3/T4 Ratio and Insulin Levels in Patients with Thyroid Disorders

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Thyroid hormones play a role in metabolism and hence affect glucose homeostasis. Both hypo and hyperthyroidism are associated with varying forms of insulin resistance (IR), which could lead to the development of Type 2 Diabetes Mellitus. A reduced T4 to T3 conversion in hypothyroid patients indicate an alteration in Type 2 deiodinase enzyme (DIO2) activity. The study included 160 patients and 31 normal controls with age range of 30 to 60 years. The patients were further divided into 4 groups namely Euthyroid (EU) ($n = 51$), untreated hypothyroid (HYPO) ($n = 11$), hypothyroid on eltroxin (HYPO-ELT) ($n = 71$) and hyperthyroid on antithyroid treatment (HYPER) ($n = 27$). None of the patients had any history of non-thyroidal illness (NTI) or any anomaly leading to LTS. Total T3, total T4, and insulin levels were determined by Radioimmunoassay (RIA) kits, while TSH was measured by sensitive IRMA assay. The insulin levels were significantly higher in HYPO group ($p < 0.001$) followed by HYPO-ELT group ($p < 0.01$) as compared to control group. The mean T3/T4 ratio was significantly elevated in HYPO ($p < 0.001$) as compared to control or euthyroid group. Further, a small subset i.e. 26% of HYPO-ELT showed significant lowered T3/T4 ratio ($p < 0.01$) when compared to normal controls. Evaluation of altered T3/T4 ratio and increased insulin levels may further help in better management of treatment and the related complications in thyroid disorder patients.

P081

Relationship Between Serum Insulin, Total Testosterone Levels with Total Prostate Volume in Benign Prostatic Hyperplasia

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Benign prostatic hyperplasia (BPH) is highly prevalent disorder in older men, represents a substantial challenge to public health and its etiology is multifactorial; some studies have reported positive correlation between diabetes and prostate size in BPH.

The objective of the study was to evaluate the correlation between insulin levels, insulin resistance, and total testosterone with prostate size in BPH cases without any metabolic disorders. In this study 42 symptomatic BPH cases and 38 healthy controls were included. Fasting blood was collected and total prostate volume was measured using Ultrasound for all subjects. Fasting serum glucose and insulin levels were determined by enzymatic and ELISA methods respectively. Total testosterone levels were quantified using chemiluminescence and insulin resistance was calculated by Homeostasis model assessment- Insulin resistance (HOMA-IR), Quantitative insulin sensitivity check index (QUICKI). Fasting insulin and HOMA-IR were significantly higher in the BPH group

(8.84 ± 4.7, 2.1 ± 1.1) compared to controls (7.19 ± 4.7, 1.7 ± 1.2); ($P < 0.05$, $p < 0.02$ respectively). QUICKI was significantly lower in BPH (0.35 ± 0.03) than controls (0.37 ± 0.04; $P < 0.01$). No difference in the levels of serum total testosterone was found between BPH cases (480.6 ± 162.5) and controls (530.4 ± 175). Significant positive correlation was seen between prostate size and insulin levels among BPH cases ($r = 0.36$, $P < 0.01$), however no significant correlation found between prostate size and HOMA-IR in BPH group ($r = 0.27$, $P = 0.08$). The results of the present study suggest that the hyperinsulinemia could serve as a risk factor for the enlargement of prostate size during the BPH complication. Further studies are needed to elucidate the possible role of increased insulin levels in the etiology of BPH.

P082

Role of First Trimester Maternal Serum Pregnancy Associated Plasma Protein A with Adverse Outcome of Pregnancy

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Although the primary aim of first trimester screening is to identify pregnancies at risk of aneuploidy, first trimester findings such as Pregnancy associated plasma protein-A(PAPP-A) and free β-human chorionic gonadotropin (β-hCG) may give insight into other adverse pregnancy outcomes. Studies have shown that decreased levels of serum PAPP-A is associated with adverse pregnancy outcomes. This study is done to compare the serum PAPP-A levels with the outcome of pregnancy like Premature Rupture Of placental Membrane (PROM), Intra Uterine Growth Retardation (IUGR) and Fetal distress. The objective was to compare serum PAPP-A levels with the outcome of pregnancy like PROM, IUGR and Fetal distress. After obtaining approval from Institutional Ethical committee, 224 pregnant women (group 1–83 without complications, group 2–141 with complications) were included for the study. Further group 2 was subdivided based on age:-group 2a age <30 years, group 2b age >30 years. Serum PAPP-A levels were measured by immunoassay method. Median values of PAPP-A were found to be decreased in group 2. Similar results were found in group 2b. The values of PAPP-A were decreased significantly in patients with PROM, IUGR and fetal distress. A low serum PAPP-A level may be the descriptive of poor early /abnormal placentation as the root cause. Serum PAPP-A is a marker for poor pregnancy outcome and would benefit by early identification of adverse outcome of pregnancy.

P083

Correlation of Levels of Serum Nitric Oxide with Dual Markers-a Pilot Study

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Nitric oxide (NO) is a free radical. It is a major paracrine mediator and important regulatory agent in various female reproductive processes, such as ovulation, implantation, pregnancy maintenance, labor and delivery. This study was done to assess the correlation of nitric oxide with free β-hCG, PAPP-A and age. The objective of the study was to estimate serum nitric oxide levels in first trimester pregnancy and to correlate these values with serum free β-hCG, PAPP-A and age. After obtaining approval from Institutional Ethical committee, serum samples (n = 92) collected for first trimester screening were used for the study. Serum NO was measured using Griess method. Free β-hCG, PAPP-A were measured by immunoassay method (cobase601analyser). The study subjects were divided into 2 groups based on age:-group 1 age <30 years; group 2 age >30 years. The study showed that Median values of NO, free β-hCG, PAPP-A were found to be elevated in group 2 compared to group 1. NO with dual marker showed positive correlation. Free β-hCG plays a role in NO release by inducing phosphorylation of NO synthase, thus elevated serum NO levels. PAPP-A inactivates IGF-1 binding proteins that raise serum IGF-1 levels which leads to elevation of serum NO levels. Further studies are required to correlate the elevated serum NO levels with increasing age.

P084

Correlation of Serum Oestrogen & Serum TSH levels in Post Menopausal Subclinical Hypothyroid Women

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Subclinical Hypothyroidism is defined as Elevated Serum TSH Level {6.8mIU/ml} with Normal Total T3, T4 Level. Normal Serum Estrogen in Fertile Period is 32 To 250 pg/ml, but in Menopausal Women it is 15 to 60 pg/ml. Aim of the study was to determine percentage of post menopausal women suffering from subclinical hypothyroidism and its correlation between serum estrogen and serum TSH level. Hospital based cross sectional study was performed on 100 post menopausal women attending OPD of gynaecology and medicine of SGRR. Serum estrogen, T3, T4, TSH were assayed using ELISA immuno assay method. 20 normal healthy females of age group 20 to 40 were taken as control. The study showed that 21% of post menopausal women were suffering from subclinical hypothyroidism, out of which 8% of women of age group 45 to 55 had serumTSH level[8.24 + 1.3mIU/ml] and serum estrogen level was [35.88 = 2.8 pg/ml] remaining 13% of women above 55 years had serum TSH level [9.56 + 1.57 mIU/ml] and serum estrogen level [20.46 + 2.84 pg/ml]. 20 normal healthy females of age group 20 to 40 had serum TSH level [2.12 +.57mIU/ml] and mean serum estrogen level [199.85 + 7.57 pg/ml]. Thus the study concluded that there is Serum TSH level rise with fall in serum estrogen level after attaining menopause.

P085

Study of Thyroid Dysfuction in Infertile Women

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Thyroid disorders have a great impact on fertility in both the sexes. Adequate levels of circulating thyroid hormones are of primary importance for normal reproductive function. The objectives were to

study thyroid profile in primary infertile women, the levels of serum prolactin in hypothyroid cases, to compare thyroid profile and serum prolactin levels in primary infertile women. A total of 200 subjects comprising of 100 primary infertile women as cases and 100 ages matched healthy euthyroid fertile women as control were included in the study. All assays were measured on chemiluminisence. The study showed 41% of the infertile women were having thyroid dysfunction, out of which 20% were subclinical hypothyroid, 12% were overt hypothyroid and 9% were hyperthyroid. 6.9% of infertile women were having elevated levels of both TSH & PRL. The study indicates strong association of thyroid dysfunction in infertility.

P086

Gene Expression of Mitochondrial Pathway in Male Fertility

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It is well documented that many factors may impair male fertility including, endocrine disruptors, genetic and congenital factors, post-testicular obstruction, vascular abnormalities and antispermatogenic agents. The local regulatory control is supported by a large number of inflammations such as Bcl-2, Cytochrome C, Caspase and procaspase. The objective of the study was to investigate the gene expression Bcl-2, Cytochrome C, Caspase and procaspase in infertile subjects for their relationship to sperm quality and cell death parameters. We undertook gene expression on a total of 300 individuals, including 120 fertile donors as controls and three subgroups of infertile men, normozoospermic (idiopathic unexplained; $n = 60$), oligozoospermic ($n = 60$) and asthenozoospermic ($n = 60$). These participants were selected from Departments of Urology, K.G's Medical University, Lucknow, India. We used quantitative real time PCR (qPCR) with lightCycler Fast Start DNA PLUS SybrGreen kit for Bcl-2, Cytochrome C, Caspase and procaspase mRNA and their relation to male fertility. We found decreased sperm motion kinetics was associated with decreased Bcl-2 and procaspase expression in oligozoospermic and asthenozoospermic subjects. However, cytochrome c expression was significantly increased in the oligozoospermic and asthenozoospermic infertile subjects compared to healthy fertile subjects. The study showed that Bcl-2, Cytochrome C and Caspase gene expression were altered in men with impaired fertility possibly via their associations with sperm count, motility and morphology.

P087

Functional Characterisation of Mutations in Congenital Adrenal Hyperplasia Patients (CYP21A2 Gene)

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Congenital Adrenal Hyperplasia (CAH) is autosomal recessive disease, with a wide range of clinical manifestations from severe classical form to late onset form. Hormonal measurements and mutation analysis of CYP21A2 gene is potentially important tool in diagnosis of steroid 21-hydroxylase deficiency as well as genetic counseling. Our aim was to study the functional consequence of the novel/rare mutations identified in the CYP21A2 gene. Most of the patients carry the common mutations which had been transferred from the pseudogene to the functional gene but it has been reported that an increasing number of novel/rare mutations occur in the CYP21A2 gene. Clinical and hormonal evaluations were used to categorize the patients in Salt Wasting (SW), Simple Virilizing (SV) and Non Classical (NC) forms. About 95 % of mutant alleles have apparently been transferred from linked pseudogene (CYP21P) to active gene (CYP21A2). Molecular analysis of CYP21A2 was performed in 55 patients for detection of novel/rare mutations. Novel mutations were identified by SSCP technique and subsequently sequencing of amplified product. Functional implication of novel mutations was analysed by construction of mutant plasmids by site-directed mutagenesis and subsequently by their in vitro expression in COS-7 cells. Mutation severity was assessed by calculating enzyme activity, their kinetic constant, activation energy and the protein expression of respective mutants. Homology modeling was done with the help of PDB-Swiss viewer. Mutation severity prediction softwares namely PROVEAN, SIFT and polyphen were used to predict the effect of novel mutations on 21-hydroxylase enzyme. Disease causing mutations were identified in patients comprising SW($n = 14$), SV($n = 26$) and NC($n = 15$). H365 N, F306 V, P357P, D234D are novel mutations in CYP21A2 gene. Each novel mutation was present at frequency of 1.8 % except F306 V which had frequency of 3.6%. H365 N mutant, F306 V mutant and F306 V, F306 + T i.e. double mutant were used to transfect the COS7 cells. H365 N mutant retained 63.23 % enzyme activity, F306 V mutant had 46.13% activity whereas double mutant exhibited 42.93% activity as compared to wild type enzyme for 17OHP as substrate. H365 N mutant had 64.17 % activity, F306 V mutant had 57.77 % activity whereas double mutant exhibited 52.83% activity as compared to wildtype enzyme for progesterone as substrate. Kinetic analysis revealed significant change in Km and Vmax of mutants as compared to wildtype enzyme. Activation energy as calculated by the Arrhenius equation was found to be increased in case of mutants as compared to wildtype. Protein expression of the double mutant was least as compared to wildtype protein. Homology modeling revealed that the mutation causes disturbances in protein function due to hinderances in interactions of the protein. Polyphen-2 predicts that F306 V and H365 N are probably damaging mutations. PROVEAN assigns H365 N and F306 V as deleterious mutations. SIFT predicts that both the novel mutations affect protein function as a result of substitution of amino acid at the respective positions. This is a comprehensive study showing the functional consequence of the novel/rare mutations in CYP21A2 gene. These novel/rare mutations were harboured by the non-classical patients and data exhibited by various assessed parameters also predicts that these mutations are close to the non-classical phenotype.

P088

Influence of Iron Status on Iodine Utilization and Thyroid function in the Hilly and the Plain Regions of Eastern Nepal

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Iodine and iron deficiency presents a major public health problem in Nepal with 19.4% of its 27 million people being at risk of iodine deficiency and 48% of total school children are suffering from iron deficiency anemia. Iron deficiency mainly manifested in the form of iron deficiency anemia is due to lack of adequate iron and vitamins in diet and hook worm infestation, though other factors may also cause it. Iodine deficiency is mainly attributed to low levels of iodine content in the diet. The objective of the study was to explore the effect of iron status in utilization of Iodine and thyroid function in hilly and plain regions of Eastern Nepal. A community based cross section study was conducted among 489 primary school children (52.14% boys and 47.86.0% girls) from Bhojpur (Hill) and Jhapa (Plain) districts of Easten Nepal. Hemoglobin, Iron, TIBC and thyroid function parameters (fT_3, fT_4 and TSH) were estimated by commercial test kits. UIC and SIC were measured by APDM and RTK method respectively. Data were expressed in frequency, percentage, Mean ± SD and median (IQR) according to the nature data. Chi-square test, Independent't' test, Pearsons and Spearman's correlation and Mann-Whitney U test were applied to test the significance considering $p \leq 0.05$ at 95% confidence interval. The Median UIC of Bhojpur was significantly lower as compared to Jhapa (147.35 (83.6, 261.85) μg/L vs 363.31 (219.04, 481.5) μg/L, $p = 0.001$). Iron status parameters: Iron and TIBC were significantly different between SAC of Jhapa and Bhojpur district (Iron; 98.33 ± 43.86 μg/dL vs 74.32 ± 32.0 μg/L, $p = 0.0001$, TIBC: 260.60 ± 76.23 μg/dL vs 376.65 ± 41.92 μg/dL, $p = 0.0001$) but hemoglobin was not significantly different (13.62 ± 1.62 g/dL vs 13.10 ± 1.43 g/dL, $p = 0.052$). Thyroid function parameters fT_3 and fT_4 were significantly different in SAC of Jhapa and Bhojpur (fT_3; 3.07 ± 0.56 pg/mL vs 2.90 ± 0.50 pg/mL $p = 0.050$, fT_4; 1.14 ± 0.18 ng/dL vs 1.37 ± 0.23 ng/dL, $p = 0.0001$), but TSH was not significantly different. TIBC was significantly negatively correlated with, fT_3 and UIC ($r = -0.182$, $p = 0.007$ and $r = -0.208$, $p = 0.002$). Thus the study showed that iron status has influence at the functional level of iodine rather than its nutritional level.

P089

Reverse Phase Column Chromatography (RPCC) for High Speed Separation of Amino Acids and its Derivatives within Basic and Limited Resource Poor Settings

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Amino Acid Analysis is commonly used in proteomics for suspected testing for IEM, as a powerful technique to discover all the 24 Amino Acids in the plasma/urinary samples. A short turnaround time is now feasible for analysis and with very low sample volume. Objective was to study was the use Reverse Phase Column Chromatography (RPCC) for high speed separation of amino acids and its derivatives within basic and limited resource poor settings. Reverse Phase Chromatography employsa highly sensitive method, maximising laboratory productivity, inspite of newly established, restricted and limited resources available this can be achieved. Improved runtime, column longevity and ruggedness of the previous methods employed so far has to be focussed on. A new methodology using recently developed columns with an engineered particle size of 1.8 micron particles was developed on 1220 Agilent System using only a Binary pump and Variable Wavelength Detector (VWD) using UV: 338 nm 10 nm Band Width (for OPA Amino Acids):262 nm 16 nm Band Width Reference: 324 nm, 8 nm Band Width (for FMOC Amino Acids) The columns used were Eclipse AAA which can withstand a high pressure of 400bars HPLC. Mobile Phase Mobile Phase A: 10 mM Na_2HPO_4: 10 Mm $Na_2B_4O_7$, Ph 8.2. Ph was adjusted to 1 N NaOH with very tiny drops until 8.2 were reached. It was filtered twice through the membrane filter attached to the Membrane filter assembly. Mobile Phase B: Acetonitrile methanol: water (45: 45; 10 v/v). All mobile phase used were HPLC grade (extra pure). It was also filtered through the 0.22 micro filter paper before use. The results showed a 30 Minute runtime was given for each calibrator, control and sample. All 24 Amino Acids were calibrated and the graph obtained. As a pilot run we checked with 19 samples (15 plasma and 04 urine samples of New Born and Infant Age Group) was run and calculated the AUC and the actual concentration of AA present. The study showed this rapid and robust way of measurement of the amount of Amino Acid in Plasma and Urine Samples utilizing minimal resources with customised timetable worksheet helped to attain good calibration curved provides the best assessment tool. Although the convention detector used is Diode Array Detector (DAD) or Fluorescent detector we could accomplish the calibration of all 24 Amino acids and sample analysis was satisfactory.

P090

Effect of Storage on Various Biochemical Parameters in Human Serum Samples Stored at Ambient Temperature (22 to 24°C) at Different Time Intervals

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In a medical laboratory post-testing, non-separated human serum samples are stored at ambient temperature (22–24°C) for 24 hours so that it may be used for re-testing, additional testing or as quality control (QC) sample for precision check. It will be interesting to see if any biochemical variation takes place in such serum stored in above conditions. Study aims to find the effect of storage on various biochemical parameters in stored samples. Five samples were centrifuged at 4000 rpm for 10minutes. The non-separated centrifuged serum was tested at intervals of 2,4,6,8 and 24 hours for various biochemical parameters viz. urea, uric acid, creatinine, protein, albumin, alkaline phosphatase, total bilirubin, AST, ALT, sodium, potassium, chloride, calcium, magnesium, phosphorus, LDH on fully-automated Siemens biochemistry analyzer "Dimension RXL-Max". During the whole procedure the samples were kept at ambient room temperature (22–24°C). Percent coefficient of variation (%CV) for all parameters were calculated and compared with the target CV of the laboratory. The results showed percent CV obtained for various parameters were as follows: urea (3.6), uric acid(2.3), creatinine (6.7), protein(0.7), albumin(2.4), alkaline phosphatase(1.7), total bilirubin (9.0), AST(5.0), ALT(2.2), sodium(1.0), potassium(1.4), chloride(0.8), calcium(1.6), magnesium (1.8), phosphorous (2.8), LDH (1.6). No obvious trend was observed in the values obtained. The study showed that low %CV is a reflection of a good laboratory practice and better internal quality control measures. However low %CV and no trend in the graphical study suggest that centrifuged non-separated human serum sample remains stable for

biochemistry parameters at ambient temperature for 24 hours. A further study with more number of samples is recommended.

P091

Use of Biological Variation in Laboratory to Set Quality Goals

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A new process control tool based on biology can quantify "allowable" imprecision and bias. Imprecision and bias contribute to overall error in a patient test result. Too much imprecision and/or bias can result in a grossly inaccurate patient result leading to misdiagnosis, incorrect prognosis, or an inappropriate treatment plan. Use of biological variation provides an opportunity for the laboratory to define and limit how much imprecision and bias are allowable based on biology and not purely statistical model. Such a process control tool provides a powerful adjunct to traditional process controls. It is not intended to replace traditional schemes but to support them. The objective of the study was to study the use of Biological Variation in Laboratory to set Quality Goals. In our study we have taken Bias, Imprecision and Z score as tools to calculate laboratory total error. To calculate allowable total error used CLIA guideline to calculate minimum, desirable and optimum performances. TE = z score × (imprecision,%) + |bias,%|, BIAS: ((lab mean – group mean) / group mean) x 100 = lab bias,%, TE(allowable) = z score × (target imprecision,%) + |target bias,%|. In the current study the following are the observations. We have observed 43 parameters on Beckman Coulter DXC860i & all are performing well in terms of CV which is 1.9 – 14.2 with in the acceptable limit. With BV 20 parameters have optimum performance,14 are below optimum performance, 6 are below desirable performance and 3 are below minimum performance. With the above observation we can conclude that, 1. IQC monitors the analyzer performance and the day to day performance of the process, 2. EQAS monitors the performance of the laboratory in terms of bias, 3. BV will help to set performance goals to achieve the good practice in the laboratory, 4. BV can be evaluated once in 6 months since CV includes all errors, 5. Checking the lab performance parameter wise with BV will be helpful for the laboratory to eliminate systemic errors.

P092

Comparison of Quality Control (QC) for Total Calcium by O-Cpc and Nm-Bapta Methods on Roche Cobas Integra 400 Plus

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Quality can be roughly defined as an attribute when met with customer's needs, should satisfy them. It is subjective with respect to both individuals and time. Thus improving quality is a continuous process. Reliability and validity concepts provide good objectivity to the term 'Quality'. Due to change in diseases vector, importance of accurate lab results and thereby QC methods is well recognized in last decade. A new method viz. NM-BAPTA was developed by Roche to estimate Total Calcium. This could have several advantages over the old method viz. O-CPC, including improved linearity, better accuracy and increased stability. The aim of the study was to evaluate QC performance of total calcium by NM-BAPTA and O-CPC method on Roche CobasIntegra 400 plus. Monthly data of Internal and external QC from January 12 to December 13 (O-CPC) and January 14 to August 15 (NM-BAPTA) on Roche Integra 400 plus (007560) was compared for Coefficient of Variation (CV) and per month calibrations. Box plot and t-test was used to depict the results. The results showed that the mean (SD) CV by O-CPC method was significantly greater compared to NM-BAPTA method [3.40(1.13) vs. 2.08(0.84), $p < 0.001$). Mean (SD) calibrations required per month by O-CPC method were significantly greater compared to NM-BAPTA method [3.63(1.84) vs. 0.90(0.72), $p < 0.001$). Box-plots suggested that not only the central value but also the variability was less with NM-BAPTA method. The study concluded that the QC performance of NM-BAPTA over O-CPC method in terms of CV is indicative of better precision by NM-BAPTA. The same was confirmed through comparison of mean calibrations required per month.

P093

Use of Urinary Protein Creatinine Index in Spot Urine Sample in Type 2 Diabetes Mellitus Patients with More Than 10 Years of Diabetic History

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Microproteinuria describes the presence of protein between 30 and 300 mg per 24 hours in the urine. Detection of an increase in protein excretion is known to have both diagnostic and prognostic value in the initial detection and confirmation of renal damage. Microproteinuria is a major component of long term complications of Diabetes Mellitus. Urine protein measurement after 24-hour urine collection is a time consuming traditional standard method for the detection of proteinuria. As an alternative, random spot sampling for a urine protein to creatinine index (PCI) has been investigated. The aim of the study was to determine the PCI for prediction of significant proteinuria with the evaluation of the P/C index in type 2 diabetes mellitus patients with more than 10 years of diabetic history. Fifty patients having more than 10 years of diabetic history who fulfilled the criteria comprised the study group and fifty non diabetic healthy subjects were taken as control group. Spot urine samples for measuring urinary PCI were obtained. Pyragallol red colorimetric method was used to estimate urinary protein. Modified Jaffe's alkaline method was employed to estimate urinary creatinine. The urine protein: creatinine index was calculated by dividing the urine protein concentration (mg/L) by urine creatinine concentration (mmol/L) multiplied by 10. Protein: creatinine index was found to be significantly higher in diabetic subjects (909.19 ± 250.06) as compared to the control group (136.43 ± 46). There was no diabetic subject with frank proteinuria estimated qualitatively. The present study suggests that random urinary PCI can be a good predictor of significant proteinuria in long term patients of diabetes mellitus. In the absence of frank proteinuria in diabetic subjects, PCI can be used to evaluate the presence of renal damage. This test could be an alternative method to the 24 hrs urinary protein estimation.

P094

Biochemical Parameters in Women with Polycystic Ovary Syndrome below 20 Years of Age

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age, affecting 4–18% of these women. It has significant clinical and biochemical sequelae; including reproductive features (such as infertility, hyperandrogenism, and hirsutism) and metabolic derangements (such as obesity, insulin resistance, impaired glucose tolerance, type II diabetes mellitus, and adverse cardiovascular risk profiles). The objective of present study was to measure levels of biochemical parameters in women diagnosed with PCOS. Young women below 20 years of age, diagnosed with PCOS (N = 65), not on any treatment, attending OPD of Obstetrics and Gynecology at Ashvini Naval Hospital, Mumbai were included in the study. Biochemical parameters were measured using standard procedures. Laboratory normal reference ranges were used for comparison. The study showed 54 % of the women with PCOS were overweight or obese according to the BMI and 55% had waist circumference >88 cm. Levels of Fasting and Postprandial Glucose, Insulin and HOMA-IR were within the normal reference ranges indicating that no Insulin resistance was seen in these women. 38%of the women had a serum total Cholesterol level above 200 mg/dL. HDL cholesterol was lower than the desirable value. Serum Triacylglycerol was within the normal reference range. SerumTestosterone, Estradiol, Prolactin and TSH were found to be within the normal reference ranges. The study concluded that no significant Insulin resistance and hormonal abnormalities were observed, Dyslipidemia was observed. These findings differ from reports in literature where Insulin resistance and Hyperandrogenism have been associated with PCOS.

P095

Effect of Delayed Sample Processing & Storage of Serum on Estimation of Thyroid Stimulating Hormone

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The significant time elapses between the collection and processing of the sample due to constraints of manpower and infrastructure may lead to discrepancy in lab results. The possible discrepancy arising out of delayed sample processing and storage was quantified in this study. The objective was to study the effect of temperature and time on the estimation of TSH in serum. Whole blood samples of 10 ml each were collected from 105 subjects. Each sample was divided into four aliquots, 1st aliquot of 4 ml and subsequent three of 2 ml each. The serum was separated following standard protocol. The TSH estimation was done by chemiluminescent immunometric assay on Immulite 1000 system. The serum TSH of 1st aliquot was labeled as zero hour value. The remaining serum after 1st analysis was divided into three aliquots and stored at 2–8°C. The stored serum was analyzed subsequently at 24 hr, 48 hr and 72 hr. The three aliquots of whole blood, stored at room temperature were processed at 2hr, 4hr and 6 hr after initial collection and analyzed. the study showed that the whole blood sample stored at room temperature for 2hr, 4hr and 6hr showed no significant difference in TSH values compared to zero hour value. TSH values remained statistically stable in stored serum upto 72hrs at 2 to 8°C. The study concluded that the TSH estimation can be done in whole blood sample separated after a delay of six hours stored at room temperature and in serum up to 72 hours stored at 2–8°C.

P096

Study of Urine Microalbumin Levels in Non-Diabetic Hypertensive Patients

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Hypertension is common, asymptomatic readily detectable disease that leads to lethal complications like stroke, myocardial infarction and renal disease. Microalbuminuria is defined as an increase in albumin excretion rate within a specific range of 30–300 mg of albumin/day. It's one of the earliest indicators of kidney injury in hypertension. The study was aimed to determine urine microalbumin levels in non-diabetic hypertensive patients. A cross-sectional study was conducted in 100 diagnosed cases of hypertension of age group between 35 – 60 years attending Medicine OP at SRMMCH & RC & equal numbers of healthy controls were selected. Fasting blood samples were analyzed for glucose, lipid profile, urea, creatinine, sodium, potassium and urine sample for microalbumin were obtained and analyzed in AU-400 auto-analyzer. All the statistical analysis was performed using SPSS 21.0. The study showed a urine microalbumin level was significantly higher in hypertensives as compared to controls (mean ± SD 44.70 ± 40.05 Vs 13.42 ± 5.68 respectively, $p < 0.0001$). Serum urea, creatinine and sodium were significantly higher in hypertensives than controls, while no significant difference was observed for potassium. Total cholesterol, triglycerides & LDL-C were higher & significant decrease in HDL-C in hypertensives than controls. The study concludes that elevated levels of urine microalbumin, along with lipid profile, urea, creatinine & sodium in hypertensives can be a determinant of cardiovascular & renal risk.

P097

Do PSA Levels Change with Age?

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A higher than normal level of PSA in blood does not necessarily indicate prostate cancer. There are several non-cancerous reasons, enlarged, infected or diseased prostate for elevated PSA levels. As men age, their prostates tend to become enlarged, even in the absence of any type of prostate related disease. Healthy prostates naturally produce small amounts of PSA and as the gland enlarges it typically produces more PSA. Therefore, one of the causes for elevated PSA level is age related prostate enlargement. Because of this fact we, at P D Hinduja Hospital, have developed an age-based threshold level of PSA in 1318 apparently healthy men in the age group of 31–80 years, coming to our hospital for a routine health

check. Blood samples were analysed for PSA using chemiluminescence enzyme immunoassay with sensitivity of 0.003 ng/ml. The subjects were divided into 5 groups of 10 years age intervals for statistical analysis. We observed a gradual increase in PSA levels with advancing age with maximum of 1.81 ng/ml in 31- 40 years age group to 5.2 ng/ml in 71–80 years. Although, traditionally 4 ng/ml has been used as the cut off for concern about risk of prostate cancer, our study suggests that use of age specific PSA threshold along with digital rectal examination may increase the clinical sensitivity and specificity of the tests and may help clinicians to rule in or rule out any further invasive procedures. It is therefore important to bear in mind that PSA levels do change with age and that PSA is prostate gland specific and not always prostate cancer specific.

P098

Study on Serum Gamma Glutamyl Transferase Levels in Obesity

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Glutathione (GSH) is the major intracellular non-protein thiol defense against free radicals. GGT is utilized by cells for the *de nova synthesis* of intracellular GSH. Elevated GGT is strongly associated with obesity and excess deposition of fat in liver, termed NAFLD, which is thought to cause hepatic IR and to contribute to the development of systemic insulin resistance and hyperinsulinemia. Thus, GGT might reflect metabolic alterations and could serve as a marker of the insulin resistance syndrome. The aim of the study was to evaluate the serum GGT levels in obese individuals. A cross-sectional study was conducted in 100 obese individuals of age 18 – 45 years, they were recruited from MHC at SRMMCH & RC and equal numbers of Non-obese controls were selected. Plasma samples collected for Glucose (FBS, PPBS). Serum samples collected for RFT, FLP and LFT were obtained and analyzed in auto-analyzer. All the statistical analysis was performed using statistical package SPSS. The study showed that serum GGT levels in obese individuals were significantly increased as compared to controls. Serum level of cholesterol, Triglycerides, LDL-C & VLDL were significantly increased, and significant decrease in HDL-C in obese individuals compared to control. Serum levels of Total protein shows significant increase in obese individuals as compared to controls. The study concludes that elevated levels of GGT in obese individuals can lead in while increase of stress.

P99

Serum Uric Acid: A Non Invasive Indicator for COPD Severity

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Uric acid (UA), a major antioxidant is present in significant amounts in the respiratory tract, providing first line of defense against environmental oxidants. This molecule has a protective role in respiratory diseases like chronic obstructive pulmonary disease (COPD). However, recent studies have highlighted the proinflammatory nature of the molecule. Therefore, the role of UA in COPD is not well characterized. The objective of the study was to find the association of serum UA in COPD patients and correlate it with spirometric parameters, oxygen saturation and the inflammatory marker, CRP. 60 COPD patients attending the OPD /IPD with the medical history, demographics, clinical parameters were recorded. Blood samples were collected prior to initiation of treatment for renal and lipid profile, UA and CRP estimation. The diagnosis and classification of airflow limitation was based on post bronchodilator spirometry in stable condition according to the Global initiative for chronic obstructive lung disease(GOLD) criteria, supported by spirometric evidence of airflow obstruction (Forced expiratory volume in 1sec (FEV_1)/ Forced vital capacity <0.70). The study showed that UA levels were significantly higher in patients with more airflow obstruction- GOLD stages III & IV, than in the milder subgroup-GOLD stages I & II ($p < 0.05$). A significant positive association of serum UA with CRP values in patients with severe airflow obstruction ($p < 0.05$) was observed. Patients with higher UA were more dyspnoeic, had associated cardiovascular comorbidities, required prolonged hospital stay and non-invasive ventilation therapy. The findings in our study highlight the possible role of UA as a biomarker of disease severity and may identify patients with worse prognosis.

P100

Population Based Biological Reference Intervals (BRI) for PSA and Thyroid Parameters (T_3, T_4, TSH) in Indian Population – A Pilot Study

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ABRI is usually defined as the set of values 95% of the normal population. Our aim in this study is to estimate BRI for blood biochemical parameters in an adult (18–60 yrs) Indian population. Samples of 300 voluntary blood donors from various geographical areas of India were analyzed on Architect plus I 1000 SR fully automated immunoassay analyzer. In our study for PSA we established the BRI as per the age group and for thyroid parameters gender wise. In the study BRI for PSA for age group 18–30 years is 0.003 to 2.559 ng/ml, 31–40 years is 0.008 to 1.745 ng/ml, 41–50 years is 0.005 to 2.974 ng/ml and 51–65 years is 0.241 to 1.963 ng/ml. BRI for T_3 in males is 0.81–1.50 ng/ml and in females it is 0.82–1.43 ng/ml, T_4 in males is 4.83–8.88 ng/ml and in females it is 5.0–9.31 ng/ml and TSH in males is 0.52–5.27 ng/ml and in females it is 0.61–4.99 ng/ml. The study showed that as compared to the published data (0–4.0 ng/ml) in our Indian population the PSA BRI is low. The lower range of BRI at age of 51–65 years is higher as compared to lower age groups, this could be age related. Hence the BRI can be kept as 0.2 to 2.0 for higher age groups in Indian population. BRI for thyroid parameters in males and females in Indian population and published parameters do not show much variation.

P101

Optimization of HPLC Based Method for Measuring Physiological Amino Acids

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Amino acid quantification acids in diagnosis of metabolic defects like aminoacidopathies, organicacidurias and other small molecule diseases. Recently they have also been reported for assessment of organ dysfunction, malignancies and metabolic syndrome. For their quantification dedicated/expensive equipment like amino acid analyzer & tandem mass spectrometer have been used. Reverse phase HPLC methods with fluorescence detection have also been reported. However for physiological amino acid estimation commercial reagents with automated sample preparation units are required. We aimed to optimize an accurate, simple and rapid method for detection of physiological amino acids which can be easily adopted by routine clinical laboratories. Ophthalaldehyde derivatized plasma amino acids were separated & quantified using a reverse phase ODS 2 column and fluorescence detector. Retention time of each amino acid was identified using individual aqueous standards. The method performance was validated as per the required criteria and the reference range for 50 healthy adults was verified using matrix based RECIPE calibrator & controls. In this study separation of 22 amino acids was achieved in 55 minutes. Peak height of each amino acid in a mixture was linear upto 1300 nmol/ml with an LOD and LOQ of 70 and 50 nmol/ml respectively. A CV <20% was obtained for accuracy and precision while recovery ranged from 80 – 120%. The external PT samples of 6 cycles from ERNDIM also showed concurrent results. The study concluded that this method is optimized for separating and quantifying amino acids. It can be used for amino acid estimation in clinical conditions.

P102

Lipid Fraction Changes in Early Rheumatoid Arthritis Post Treatment

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Current evidence suggests that dyslipidemia occurs in RA and is a major CVD risk factor in patients. Anti-inflammatory drugs help reverse some of these changes and associated risk. The objective of the study was to compare the effect of 6 month treatment of naïve RA patients with disease modifying anti rheumatoid (DMARDs) therapy on lipid fractions and atherogenic indices. Forty patients of early RA, as per ACR criteria, with less than one year duration of disease were recruited with 30 healthy volunteers. Patients were treated for 6 months with DMARDs. Lipid profile, Apoproteins, Lp(a), sdLDL were done pre and post treatment and compared. The study showed that the mean DAS 28 score at disease onset was 5.15 ± 1.3. Total Cholesterol; ($p = 0.0011$), Low density Lipoprotein Cholesterol; ($p = 0.0025$), Apo B ($p = 0.0005$), Lp(a)were significantly higher with lower HDL-Cholesterol and Apo A-1 in patients. The atherogenic ratios {TC/HDL-C ($p = 0.0004$); LDL-C/HDL-C ($p = 0.0012$)} and the atherogenic index of plasma {log (TG/HDL-C)($p = 0.0276$)} were significantly higher. Post treatment the serum HDL-C levels improved with consequent improvement in atherogenic ratios {TC/HDL-C ($p = 0.0022$); LDL-C/HDL-C: ($p = 0.0012$)} and Lp(a) decreased significantly ($p < 0.0001$). The study concludes that ERA patients display an atherogenic lipid profile which is modified on treatment with the DMARDs. Atherogenic indices are better risk predictors than individual fractions. Early intervention therapy to control the disease will reduce the risk of atherosclerotic process and cardiovascular events in RA patients.

P103

Role of DNA Quantification Before PCR

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In molecular biology, quantification of nucleic acids is commonly performed to determine the average concentrations of DNA or RNA present in a mixture, as well as their purity. Reactions that use nucleic acids often require particular amounts and purity for optimum performance. There are several methods to establish the concentration of a solution of nucleic acids, including spectrophotometric quantification and UV fluorescence in presence of a DNA dye. The pre-analytical steps need standardization and quality control in order to obtain consistent and inter-laboratory reproducible results from DNA analysis. Many modern molecular biological applications's sample size has become increasingly smaller and the determination of nucleic acid concentrations from these small volumes has become an increasingly demanding task in modern molecular biology. The purpose of this study was to evaluate the DNA quantity and quality by using different methods of DNA quantification. The most popular technique for determining nucleic acid concentrations is based on measuring the absorbance at 260 nm (A_{260}). The purity of the DNA or RNA is checked by comparing absorbance values from 260 nm and 280 nm measurements (260 / 280 ratio). DNA quality assessment was done by electrophoresis. The extracted DNA was evaluated by loading 100 ng of DNA based on both NanoDrop and Qubit measurements on a 0.8% agarose gel electrophoresis; ethidium bromide stained DNA gels were evaluated by using Gel Documentation. DNA concentration on NanoDrop were found to be on higher side as compared to the concentration on Qubit after running the samples on agarose ($p < 0.005$). The study concludes that to avoid wastage of sample material, detrimental effects in terms of cost and time consumption, DNA quantification is must before proceeding for PCR. Our data strongly suggests that the ideal sequence to qualify and quantify DNA from samples is to first assess the presence of contaminants in the sample with NanoDrop, and subsequently use Qubit to quantify the dsDNA.

P104

Effect of Matrix on Commonly Measured Analytes in the Emergency Setting using the Dry Chemistry Technology

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Data on dry chemistry slide technology for serum versus EDTA plasma is unavailable. Hence, this study aimed to evaluate serum vs EDTA plasma outcomes for selected parameters in 51 healthy individuals using dry chemistry autoanalyzer (Vitros-350, Orthoclinical Diagnostics). Paired samples were processed simultaneously for Glucose, Urea, Creatinine, Uric acid, TBIL, DBIL, AST, ALT, Total protein, Albumin, Cholesterol, dHDL, Triglycerides, Sodium, Chloride, Phosphorus, CPK (total), CPK-MB, LDH, Amylase, Lipase and CRP. Data were analyzed using SPSS-16. Comparable results between serum vs plasma were obtained for Glucose (97.56 vs 99.80, $P = 0.6$), Urea (21.6 vs 20.6, $P = 0.1$), Creatinine (0.74 vs 0.72, $P = 0.5$), DBIL (0.22 vs 0.20, $P = 0.2$), ALT (50.9 vs 51.6, $P = 0.5$), Total protein(7.7 vs 7.8, $P = 0.1$), Cholesterol (163.1 vs 157.1, $P = 0.06$), dHDL(46.9 vs 46.0, $P = 0.2$), Triglyceride (150.2 vs 149, $P = 0.9$), Sodium (141.8 vs 141.3 $P = 0.7$), CPK-MB(15.0 vs 13.7 $P = 0.1$), CRP(0.72 VS 0.64, $P = 0.4$). Statistically significant differences were observed for Uric acid (5.4 vs 5.0, P = 0.01), TBIL(0.77 vs 0.65, $P = 0.001$), AST(15.3 vs 3.9, $P < 0.001$), Albumin(4.5 vs 5.0, $P < 0.001$), Chloride(108 vs 105, $P < 0.001$), Phosphorus(3.9 vs 3.6, $P < 0.001$), CPK-total(117.2 vs 43.9, $P < 0.001$), LDH(125.7 vs 92.8, $P < 0.001$), Amylase(75.9 vs 44.8, $P < 0.001$) and Lipase(130 vs 72, $P < 0.001$). This study highlights that parameters like Glucose, Urea, Creatinine, DBIL, ALT, Total protein, Sodium, CPK-MB, and CRP which are commonly done in the emergency setting can be reported from EDTA plasma samples also, in cases where serum is not available or lack of time for serum to separate.

P105

Application of Sigma Methodology in Biochemistry

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Clinical laboratory results directly impact medical decisions and hence it is required to minimize variations in test results. Sigma metrics is a management strategy focusing on improving quality of services. It measures the performance variations in terms of deviations per million in pre and post analytical steps and in sigma levels calculated from %CV and bias in analytical tests. We aimed to calculate sigma level of analytical parameters and assess our performance from January 2013 – July 2015. Sigma levels were calculated for creatinine, AST, albumin, bilirubin, potassium, Trop T, CK-MB, glucose, ionized calcium, lipase, lactate and CA 125. Sigma level were calculated using %CV of internal QC data, % bias from respective CAP proficiency testing surveys for each survey cycle received from January 2013 – July 2015. The mean sigma level for each year for individual parameters was used for assessing laboratory performance over the year. In the study the sigma level showed a wide inter analyte variability with ionized calcium as <3, lactate, potassium, CKMB, glucose & albumin between 3 and 6 and creatinine, SGOT, Troponin T, Lipase and CA 125 as >6. There was mild to significant variations over the years in some parameters. These variations were due to changes in methodology or internal controls or other conditions. The study shows that Sigma calculations have elucidated the analytes with low sigma level and has also helped us identify the causes of variations over the years. This will help to improvise laboratory performance.

P106

Comparison of Analytical Performance of Enzymatic Method vs Jaffe's Kinetic Method for Creatinine Estimation

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Clinical biochemistry laboratories use several methods for the estimation of serum and urinary concentrations of creatinine. With the recent introduction of the reporting of estimated glomerular filtration rate (eGFR), inter-laboratory agreement of serum creatinine results has become an important international priority moreover expert professional bodies have recommended that all creatinine methods should become traceable to a reference method based on isotope dilution-mass spectrometry (IDMS). The major analytical problems associated with estimation of creatinine are positive and negative interference by chromogens. Though enzymatic creatinine assay is widely accepted as one of the most accurate routine methods anddeals effectively with most interfering substancesit has a greater cost and shorter shelf-life compared with the kinetic Jaffe's method. Hence this study was taken up to compare the analytical performance and practicability of the Enzymatic method to Jaffe's kinetic method in estimation of Urine and Serum creatinine. The study was conducted over a period of 8 months. The study group consisted of 72 healthy male volunteers aged between 18 and 28 yrs of Sri Ramachandra Medical College & RI participated in this study. Serum samples and urine samples were collected from the volunteers. Creatinine was analyzed both by kinetic Jaffe's and enzymatic method in DAYTONA fully automated analyzer using the commercial kits from Randox laboratories. In the study the mean serum creatinine of the study group was 0.92 ± 0.18 mg/dl by Jaffes method and 1.07 ± 0.23 by the Enzymatic method. The mean urine creatinine of the study group was 59.87 ± 30.09 mg/dl by Jaffe's method and 77.90 ± 20.38 by the Enzymatic method. Method comparison between the enzymatic creatinine method and Jaffe's kinetic method by linear regression analysis gave a coefficient correlation R of 0.66 for serum and o.647 for urine samples. To our surprise we found that enzymatic method over estimates creatinine than Jaffe's method. An important conclusion from the study is that routine creatinine methods can have significant degrees of bias and calibrations based on non-commutable reference materials can also cause significant errors in report obtained. Thus the clinical biochemists should have a good understanding of the relative performance of routine creatinine methods.

P107

Study of Serum Magnesium and Glycated Hemoglobin in Diabetic Patients along with Changes in Their Lipid Profile

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Diabetes mellitus is one of the most prevalent diseases worldwide. Dyslipidemia is one of the major established risk factor for cardiovascular disease in diabetes mellitus. Glycated hemoglobin is indicator of glycemic status over long term. Hypomagnesaemia is a common feature in patients with type-2 diabetes. It may be a cause or consequence of diabetes. This study was designed to evaluate the correlation between HbA1c, lipid profile, serum magnesium levels in relation to their glycemic status. The present study was conducted on one hundred fifty patients in Department of Biochemistry in association with Department of Medicine, Govt. Medical college, Amritsar. These patients were classified into two groups : 75 known diabetic patients and 75 controls were taken for the study and investigated for fasting blood sugar, HbA1c, lipid profile (cholesterol, triglycerides, HDL, LDL) and magnesium. In the study the mean HbA1c, total cholesterol, triglycerides, HDL, LDL and magnesium were 8.40 ± 1.24, 203.24 ± 21.66, 173.16 ± 23.62, 38.34 ± 7.07, 130.2 ± 23.15, 1.72 ± 0.34 respectively. The findings of this study illustrate that HbA1c had direct and significant correlation with cholesterol, triglycerides, LDL and inverse correlation with HDL and magnesium. From the above findings it was concluded that increased HbA1c (predictor of dyslipidemia) and depletion of magnesium levels lead to early onset of complications of diabetes (CVD). Thus, early diagnosis of dyslipidemia and supplementation of magnesium in diabetic patients results in delaying or preventing complications.

P108

An in vitro Assay of Angiotensin Converting Enzyme (ACE) Inhibitory Activity in Methanolic Extract of *Carica papaya* on Sheep (*Ovisaries)* Tissues

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Angiotensin converting enzyme, EC 3.4.15.1, is a zinc metallopeptidase that converts the angiotensin I (inactive decapeptide) to angiotensin II (a potent vasoconstrictor), and bradykinin (a hypotensive peptide) to inactive components. High ACE activity leads to increased concentration of angiotensin II and hypertension. ACE inhibitors such as Captopril and Lisinopril play key roles in treating hypertension and maintaining the electrolyte balance. They are commonly used as they are safe and well tolerated with few side effects. Many medicinal plants active components are also used as drugs for the treatment of high blood pressure. The present study was aimed to estimate the angiotensin converting enzyme (ACE) inhibitory activity in methanolic extract of *Carica papaya* on sheep tissues. Tissue ACE activity was measured with Hippuryl-Histidyl-leucine (HHL) as substrate and the hippuric acid released was measured spectrophotometrically at 228 nm. Methanol extract of *Carica papaya* were used in the enzyme assay as inhibitor to determine their inhibitory effect on kidney, lung and testis ACE. The linearity of ACE activity of kidney, lung and testis enzyme was established with HHL as substrate for the incubation period of 30 min at 37°C. ACE activity was confirmed with specific ACE inhibitor i.e. Captopril a standard drug. ACE activity was determined in the presence of methanol extract of *Carica papaya* (10:1), this plant has inhibited ACE activity very significantly. The study showed that 25 µls of *C. papaya* leaves extract reduced sheep kidney, lung and testis ACE activity by 40%, 70% and 25%. The study concludes that the significant inhibition of kidney, lung and testis ACE activity by this plant suggests their possible role in controlling blood pressure or reduction in cardiovascular diseases. However, some medicinal plants thus can be considered as promising sources of natural inhibitors of ACE for medicine and commercial uses.

P109

Comparison of Plasma and Serum Electrolyte Levels in Patients Attending Emergency Out Patient Department

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Electrolytes are important parameter for clinical decision making and are measured by electrolyte analyser. Almost all metabolic processes are mediated by electrolytes, so variation in electrolyte concentration can cause variety of disorders. Electrolytes are measured in serum and plasma and it is important to compare them for correct diagnosis and treatment. The objective of the study was to compare sodium and potassium levels in serum and plasma. Hundred patients attending emergency OPD of PGMIS Rohtak were enrolled in this study. Venous and arterial blood samples were taken for serum and plasma measurement of sodium and potassium and samples were analysed in combiline blood gas analyser. The study showed that Mean serum concentrations of sodium (140.79 ± 9.31) and potassium (4.84 ± 0.87) were higher than plasma concentrations of sodium (135.62 ± 10.00) and potassium (3.69 ± 0.85) ($p < 0.001$ and $p < 0.001$ respectively). A positive correlation was also found with the serum and plasma values of both the electrolytes, the values are found to be 0.63 and 0.70 in sodium & potassium respectively. The study concludes that serum concentrations of sodium and potassium were higher than plasma level. In vitro release of potassium from cells and platelets during clotting increases serum potassium and lower plasma electrolyte values could be explained by dilutional effect of heparin. Conventionally, electrolyte analysis is performed on serum, so studies should be conducted in critical care centers and a correction factor need to be established which helps in determining accurate electrolyte concentration.

P110

Comparison of Serum Sodium and Potassium Levels by Blood Gas Analyzer and Autoanalyzer

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Electrolytes are necessary for proper cellular functioning and are important in making various clinical decisions as abnormal values can represent life threatening conditions. It is very essential to measure them accurately. There are various methods of electrolyte analysis which are commonly used. It is necessary to compare these methods for better patient care. The objective of the study was to compare sodium and potassium levels in serum when measured by blood gas analyzer and autoanalyser. This study was conducted in emergency laboratory of PGIMS, Rohtak, in which 200 venous and arterial serum samples were analyzed in both auto-analyzer (Hitachi Roche) and blood-gas analyzer (Combiline) for sodium and potassium levels. These two machines use different methods for electrolyte analysis. Arterial Blood Gas analyzer uses direct method while auto-analyzer utilizes indirect method. The results showed the mean concentration of sodium was 140.7 mmol/L (SD 7.14) using autoanalyzer and 136.6 mmol/L (SD 7.37) using ABG analyzer ($p < .001$). For potassium mean concentration was 4.1 mmol/L (SD 0.77) using autoanalyzer and 3.8 mmol/L (SD 0.73) using ABG analyzer ($p = .001$). The correlation coefficients for sodium ($r = 0.898$) and potassium ($r = 0.925$) were significant ($p < .001$). We conclude that there is statistically significant difference between sodium and potassium levels measured by direct and indirect method so clinician should be aware of two different methods of testing so that proper diagnosis can be made and different reference range need to be established for the two methods.

P111

Establishment of Reference Intervals of Hepatic Enzymes in Dakshina Kannada Population

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Age, sex, ethnicity, diet, physical and socio-economic conditions, affect the physiology of population. Hence it is necessary to setup reference values that are applicable to specific population rather than to borrow it from other population based studies or from literature from reagent kit manufacturers. The objective of the study was to establish the reference intervals of hepatic enzymes in Dakshina Kannada population. Cross sectional study was conducted in K S Hegde Charitable Hospital, Mangalore, parameters AST, ALT, ALP, GGT were analysed by IFCC recommended kinetic method, the sample size was 146 subjects (according to CLSI guidelines), descriptive analysis with mean and standard deviation was done. In results the reference intervals observed for men and women are respectively are as follows: AST (4–50)U/L and (10–25)U/L, ALT (15–62)U/L and (8–60)U/L, ALP (42–128)U/L and (22–135)U/L and GGT (8–66)U/L and (3–68)U/L. As the age advances there are differences in the reference intervals observed in both the sexes. The study showed a difference in the reference intervals between men and women and categorial age wise differences were also obtained.

P112

Evaluation of Serum Creatine Kinase and Lactate Dehydrogenase Activity in Hypothyroidism Patients

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Hypothyroidism is one of the most commonly occurring thyroid disorders worldwide. Muscle involvement in hypothyroidism is common with 30–80% of hypothyroid patients presenting with muscular symptoms. Myopathy may be the sole clinical manifestation of hypothyroidism in some cases with rise in serum creatine kinase activity and rise in lactate dehydrogenase levels. The aims of the present study were to determine the activities of serum creatine kinase (CK) and lactate dehydrogenase (LDH) in hypothyroidism patients, and to evaluate the relationship between CK, LDH and TSH levels. The present work was conducted in the Department of Clinical Biochemistry at Sri Aurobindo Institute of Medical Sciences, Indore. In this study serum CK and LDH were measured in 55 patients with known history of hypothyroidismand the results were compared with that of 30 healthy adults who were taken as control group. The results show that mean CK and LDH were significantly ($p < 0.0001$) increased in patients with hypothyroidism as compared to control subjects. A positive correlation was found between CK, LDH levels with TSH levels. We concluded that the significant elevation of serum CK and LDH activities indicate muscle involvement in hypothyroidism patients.

P113

Quality Control in Clinical Laboratory

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The aim of the study was to study the importance of Quality control in a clinical laboratory. Quality control is one of the most important aspects in a clinical laboratory testing. Maintaining accurate and frequent checks through quality control ensures both precision and accuracy of patient sample results. When quality control (QC) works effectively, it can find and rectify the source of error in the analytical processes of a laboratory timely before potentially incorrect results are released. In a clinical laboratory, the most common sources of error include clerical errors, technical errors, caliberation error, reagents instability and random errors. Many pre-analytical, analytical and postanalytical variables affect patient results. QC materials must be identical and tested identically to patient samples. The most common tool used to track laboratory quality control samples is the Levey-Jennings (L-J) chart. An L-J chart and Westgard Rules are frequently used to identify errors in a quality control run. Westgard Rules observe the normal distribution expected and identify standard deviations produced. Implementing Westgard rules within an L-J chart can identify violation of the rules based on control limits established for the sample tested. Most importantly, by tracking the running averages of the patient results, a laboratory can identify drift or problems with analyzer function that may not be captured by quality control testing. Addressing QC issues is critical to identification of potential errors with patient results.

Continuous monitoring of quality control testing and capturing sources of error is important to ensure accuracy of patient results.

P114

Study of Serum Uric Acid in Preeclampsia

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Preeclampsia, pregnancy induced hypertension (PIH), complicates about 5–8% of all pregnancies and is major cause of low birthweight, fetal growth restriction, preterm delivery of the child and morbidity & mortality both in child and mother. The present study was done to see the level of uric acid in preeclampsia and pregnancy induced hypertension (PIH) patients. Fifty patients diagnosed as having preeclampsia with age between 20–35 year and 50 control with similar age group were studied at index medical college indore(MP), after taking their consent. Blood samples were collected under aseptic precautions in plain vacutainer for serum uric acid estimation. Out of 50 patients, 15 were diagnosed as mild preeclampsia (MPE), 15 were labeled as severe preeclampsia (SPE) and 20 patients were found with PIH. Serum samples were analyzed for following parameter by semi-automated biochemistry analyzer. Uric acid estimation was done by uricase peroxidase method. Serum uric acid levels in preeclampsia and PIH patients were found to be significantly higher as compared to controls group ($p < 0.0001$). The observed mean serum uric acid levels in mild preeclampsia, severe preeclampsia and PIH patients the mean serum uric acid values were 7.23 ± 0.83 mg/dl, 8.59 ± 0.58 mg/dl, and 6.63 ± 0.51 mg/dl respectively as compared to control which was 4.12 ± 0.65 mg/dl. Serum uric acid levels were significantly higher in both preeclampsia and PIH patients & could be a useful indicator of the maternal and fetal complication in hypertensive patients.

P115

Detection of Urinary Vitamin Levels in Chronic Moderate Alcohol Consumers by HPLC-Q-TOF-MS and Their Reversal Effects by *Tinosporacordifolia*

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Heavy alcohol intake depletes the plasma vitamins due to hepatotoxicity and decreased intestinal absorption. However, moderate alcohol intake is thought to be healthy. Therefore, effects of moderate alcohol intake on liver and intestinal absorption were studied using urinary vitamin levels as markers. Furthermore, effects of *Tinosporacordifolia* water extract (TCE) on vitamin excretion and intestinal absorption were also studied. In the study, asymptomatic moderate alcoholics ($n = 12$) without chronic liver disease and healthy volunteers ($n = 14$) of mean age 39 ± 2.2 (mean $\pm$ SD) were selected and divided into three groups. TCE treatment was performed for 14 days. The blood and urine samples were collected on day 0 and 14 after treatment with TCE and analyzed. Alcoholics samples showed significant increased levels of γGT, AST, ALT, TGL, CHL, HDL and LDL ($p < 0.05$) but their level get down-regulated after TCE intervention. Multivariate analysis of metabolites without missing values showed an increased excretion of 7-dehydrocholesterol, orotic acid, pyridoxine, lipoamide and niacin and TCE intervention depleted their levels ($p < 0.05$). In contrast, excretion of biotin, xanthine, vitamin D2 and 2-O-p-coumaroyltartronic acid (CA, an internal marker of intestinal absorption) were observed to be decreased in alcoholic samples; however TCE intervention restored the CA and biotin levels. Vitamin metabolism biomarkers i.e. homocysteine, xanthurenic acid, etc. were also normalized after TCE intervention. Therefore, overall data depict that moderate alcohol intake is also hepatotoxic and decrease intestinal absorption. However TCE treatment effectively increased the intestinal absorption and retaining power of liver that regulated alcohol induced multivitamin deficiency.

P116

Establishment of Reference Intervals for Total Cholesterol, HDL Cholesterol and LDL Cholesterol in North Karnataka Population

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Reference intervals for biochemical parameters are used to aid physicians to interpret the results of clinical measurements. Reference intervals of Indian population are not readily available and the values in use are either borrowed from the text books and articles or insert literatures from kit manufacturers which are based on sample population other than Indians. To establish reference intervals for serum total cholesterol, HDL cholesterol and LDL cholesterol in an adult male (20–40 years) population of residents of North Karnataka. The study comprised of 130 normal healthy subjects in the age group of 20 to 40 years. Their serum samples were analysed for total cholesterol, HDL cholesterol and LDL cholesterol. Statistical analysis was performed using SPSS software package version 16.0. The calculations for reference intervals were carried out in accordance with IFCC and CLSI approved guidelines. The Mean $\pm$S.D of Serum Total cholesterol, HDL cholesterol and LDL cholesterol in mg/dl were 141.3 ± 31.21, 33.9 ± 11.60 and 74.4 ± 24.17 respectively. The results obtained showed variation from earlier studies in different parts of India. So the reference range obtained for selected population could be different. Hence development of reference range for all biochemical parameters in a selected population in more partitioned groups and larger sample size could be of significance to the physician.

P117

Uric Acid – An Unusual Finding

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We have come across a very unusual case of hypouricemia in a twenty six year old female patient. She was a walk-in patient in our hospital, and given her blood sample for routine parameters viz.

LFT, KFT, Lipid profile, hemogram and urine examinations. It was observed that her all the parameters were almost normal except serum uric acid that was 0.2 mg/dl much lower than lower limit of reference interval of 2.6 – 7.2 mg/dL. Uric acid level of her repeat fresh sample was also 0.1 mg/dL. This patient had no history of multiple sclerosis, fanconi syndrome, hyperthyroidism, myeloma, nephritis and Wilson 's disease. Hypouricemia is a level of uric acid in blood serum that is below normal. Hypouricemia usually is a benign and sometimes is a sign of medical condition. Usually hypouricemia is due to drugs and toxic agents, sometimes it is due to diet or genetics, and rarely is due to an underlying medical condition. Further genetic studies needs to be done.

P118

Study of Serum Calcium, Phosphorus, Magnesium and Vitamin D Levels in Patients with Type 2 Diabetes Mellitus

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Diabetes mellitus is the most common human metabolic disorder, and a major health concern is the severe morbidity and mortality associated with the late diabetic complications. Type II diabetes is common all over the world with an ever increasing number day by day. Consistent deficiency of Vitamin D, Calcium, Phosphorus and Magnesium increases the risk of new onset diabetes and early onset of diabetic complications in those who already have this disease. The present study was planned to estimate the serum levels of vitamin D, calcium, phosphorus and magnesium in type 2 diabetic patients of Uttarakhand. One hundred type II diabetic patients attending SMI Hospital (age: 20–65 years) of both sexes and fifty age and sex matched healthy controls were included in this study. Besides the established diagnostic parameters for diabetic patients, serum Vitamin D, Ca, P and Mg levels were measured in both the groups. The levels of vit D and Mgwere found to be significantly decreased in the diabetic patients as compared to the controls ($p < 0.05$). However, serum Ca and P levels were also found to be slightly lower in the diabetics but the difference was not found to be significant ($p > 0.05$). Vit D and Mg levels were noted to be lower in diabetics as compared to non diabetics whereas serum Ca and P levels were not affected. The decreased levels of vit D and Mg may play a role in the development of the disease and may possibly play a role in the management of diabetes.

P119

Quantification of Serum Uric Acid and C-Reactive Protein in Hypertensive Patients

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Hypertension is a common health problem in developing countries. Untreated high blood pressure leads to many degenerative diseasesincluding heart failure, renal disease and coronary artery disease. High uric acid has been reported to be an independent risk factor for cardiovascular eventsin patients affected by hypertension even after correcting associated risksfactors. Similarly elevated C-reactive protein concentration (CRP) may predict a higherrisk for future cardiovascular disease in hypertensive patients. The present study was conducted to quantify serum uric acid and CRP levels in essential hypertensive patients and to correlate their values withvarious grades of hypertension. Fifty essential hypertensive patients admitted inthe various wards of SMI Hospital, SGRRIMHS, Dehradun were included to study the role of C-reactive protein and uric acid in the development of essential hypertension. Fifty age and sex matched healthy volunteers were taken ascontrol group. Uric acid and C-reactive protein levels were estimated by usingfully automated dry chemistry analyzer (VITROS-5, 1 FS) and semi-autoanalyzer (ERBA CHEM-7) respectively. In the present study, mean serum uric acid and C-reactive protein levels were significantly higher in the study group as compared to the controlgroup. Uric acid and CRP levels correlated with systolic blood pressure in grade1and 2 but not in grade 3 hypertension. Correlation of uric acid and C-reactiveprotein levels with diastolic blood pressure was found to be significant in grade 2 hypertension but not in grade 1 and 3. It could be concluded that serum uric acid and CRP play crucialrole in the management of essential hypertension.

P120

Quality Indicators-An Aid to improve Laboratory Performance

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The clinical laboratory is the heart of all healthcare services. The patient management depends on the quality of reporting in laboratory services. The quality of a clinical laboratory is assessed by certain parameters, called Quality Indicators. The aim of the present study was to design and monitor quality indicators in an effort to improve the laboratory performance. The present study was done in the department of Biochemistry of SPS Hospitals, Ludhiana, a tertiary care hospital, from September 2014 to August 2015. The data was analyzed retrospectively over a period of one year and improvement in quality analyzed by the trend in the indicators. The indicators were classified broadly into three types: Pre-analytical, Analytical and Post-analytical. The Pre-analytical indicators consisted of hemolysed, lipemic, clotted and insufficient samples. The Analytical indicators consisted of Non-conforming Quality Controls and Equipment breakdown. The Post-analytical indicators consisted of Turnaround Time (TAT), critical value reporting, critical test reporting and urgent test reporting. The results were prepared for identification of the need of corrective action at appropriate areas for continuous quality improvement. A total of 59441 samples were processed in the department of Biochemistry in one year. The Pre-analytical phase indicators out-numbered the other types of indicators. Repeat sample due to hemolysis (0.8%) was the most common quality indicator observed during the pre-analytical phase followed by repeat sample for result confirmation (0.07%) and wrong test entry in HIS (0.06%). The most common analytical phase indicator was equipment breakdown followed by non-conforming quality controls. The most common post-analytical indicator was increased Turnaround Time

(0.17%). The reasons for increased TAT included pre-analytical and analytical phase events. Repeat sample due to hemolysis is the most common quality indicator. The Quality Indicators can be helpful in identification of the reason of deviation in quality indicators and thus improve the quality of laboratory services by taking appropriate corrective action at the right time. Quality indicators are the essential part of the continuous quality improvement process in the healthcare services.

P121

Validation of Methods Performance on Beckman Coulter AU 640 and AU 2700 for Routine Biochemistry Analytes

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Beckman Coulter AU 640 and AU 2700 are fully automated analyzers for spectrophotometric, ion-selective and immune turbidimetric determination of biochemical parameters. The objectives of the study were to analyze 31 analytes on Beckman Coulter AU 640 and AU2700, to compare the results on the two analyzers and to calculate total error and establish linearity for some parameters. This study was designed for validation of 31 analytes. It also included determination of within-run (N = 20) and between-run imprecision (N = 20), inaccuracy (N = 20) and method comparison on Beckman Coulter AU 640 and AU 2700 (N = 40). Total error was calculated for validation of the analytical process. Linearity was established for few parameters. Within-run imprecision CVs were all below 5 % for both the analyzers. Between run CVs for all analytes were below 6%. Analytes that did not fulfill requirements for inaccuracies were: cholesterol and magnesium on Beckman Coulter AU 2700. Analytes that deviated from quality specifications for total error on both analyzers were: sodium, bicarbonate, calcium, albumin and magnesium. Chloride showed deviation from quality specifications for total error on Beckman Coulter AU640 analyzers. Beckman Coulter AU 640 and AU 2700 had low CV values and had satisfactory accuracy and precision and are extremely stable. Linearity study showed extended linearity when compared with that claimed by the manufacturer. Method comparison study showed excellent correlation between both the analyzers.

P122

Retrospective Study of Specimen Rejection Rate and Impact of Intervention for Clinical Biochemistry in Oncology Set Up

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Most laboratory errors occur in the pre analytical phase. Identification of these errors and rejection of inappropriate samples can lead to increased turnaround time and can also affect patient care. The objectives of the study were to analyze sample rejection rate in clinical biochemistry, to take corrective and preventive action for samples rejected and to monitor rejection rate after intervention. A retrospective study was carried out on samples rejected over 8 months period based on the rejection rates and the type of rejections. The number of patients studied per month varied from 24,000 to 33,000 with an average of 26,958. The type of rejections evaluated were as follows: hemolysed samples, sample clotted, improperly labeled samples, payment not done, quantity insufficient, samples diluted with IV fluids, samples leaking and no barcode fixed on the sample and samples received beyond departmental acceptable time. A total of 2,15,662 blood samples were studied for rejection rates over a period of eight months. The rejection rates for the period from January to April 2015 before intervention were 0.12%, 0.178%, 0152% and 0.167% respectively. After intervention, in the form of forwarding the rejection issues to the respective areas of the hospital followed by dealing with these issues (corrective action taken) from time to time over a period from May to August 2015 the following observations were made; the rejection rates decreased considerably and was 0.139%, 0.123%, 0.116% and 0.093%respectively. The rejections due to improper labeling, no payment, insufficient quantity were also found to decrease with no rejections in August 2015. Slightly hemolysed samples were processed in the laboratory however, potassium and enzymes tests were not reported due to effect of hemolysis on these parameters. It is observed in the above study that monitoring sample rejection rate, pre and post intervention helps to complement the quality system in the laboratory.

P123

Developing Cost Effective Semi-Automated Sample Archiving System

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Modern laboratories today are using automations available in pre-analytical, analytical and post-analytical sections. In developed countries, labs use a completely automated sample archiving system for fast retrieval of patient samples. Such systems may not be feasible in labs of developing countries. Identifying this need for developing a cost effective method for sample archiving, we have attempted to develop a cost-effective strategy for easy sample archival at no additional cost for the labs. To develop a novel, cost-effective method of post analytical sample archival system. With the use of a Simple yet innovative Logic in the Microsoft Excel Sheet's structural and functional aspects, also by using some thermocol trays (used for packing vacutainer's), patient sample's and a barcode scanner along with a Computer, an integrated system was made as a standard operating protocol for the method development and the efficiency of it was analysed for a period of six months. Multiple tests were carried out which compares the total time consumed by a manual sorting method of samples & our developed method, which proves that the latter is more effective & faster than the former. Based on the analysis, it is evident that, proper archiving is a necessity for quick retrieval of samples in mid and large scale labs. It can be used in developing countries at no additional cost. This may pave way for more newer and cost effective archiving methods in future.

P124

Distinguishing Different Characteristics of Anemia by Biochemical Parameters Available in Diagnostics – Clinical Biochemistry

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Anemia is commonly caused due to iron deficiency. The most common cause of iron deficiency is heavy menstrual periods. Bleeding into the gut is a common cause in older people. To diagnose anemia, physical examination, complete blood count and a test that determines the size and shape of the red blood cellsis very important. Other tests may be ordered to determine the underlying cause of anemia. The objective is to determine the usefulness of total iron, total iron binding capacity (TIBC), ferritin, % transferrin saturation and haemoglobin in patients with iron deficiency anemia (IDA) and anemia of chronic disease (ACD). The study included 479 patients with benign and malignant tumors in the age group of 14–86 years. Total iron and total iron binding capacity (TIBC) were estimated using Beckman Coulter kits on fully automated Beckman Coulter AU640 chemistry analyzer, Ferritin assays were carried out on fully automated immunoassay analyzer Architect i2000sr system and % transferrin saturation was calculated using the formula(Iron/TIBC) *100. Complete Blood Count (CBC) was measured on fully automated CBC analyzer. In the present study, total iron, TIBC, ferritin, % transferrin saturation and CBC was examined for distinguishing iron deficiency anemia from anemia of chronic disease based on the biochemical criteria. On analyzing the data based on biochemical tests 8.77% cases had iron deficiency anemia and 33.4% had anemia of chronic disease whereas 6.79% had haematochromatosis. The remaining 50%cases needed further investigations to categorize them. The study reveals that based on the hematological analysis and biochemical findings we can group patients into ACD and IDA. Knowing the causes of development of anemia is more important in deciding the line of treatment.

P125

Estimation of Protein Creatinine Ratio in Urine Samples from Early Neonates

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The amount of protein excreted in the urine of a person depends on the age, race, and gender of a person, and is known to increase in various conditions having pre-renal, renal, or post-renal origin. Since the collection of urine over a 24-hour period is cumbersome and delays diagnosis, the protein creatinine ratio (PCR) is used to rule out proteinuria. However, a reference interval needs to be established for different age groups, genders, and races. No such reference interval has been established for Indian neonates. The objective of the study was to estimate the amounts of protein and creatinine present in random spot urine samples from early neonates and to calculate the protein creatinine ratio. Random spot urine samples were obtained from 1 to 3 day old neonates (16 females) of gestational age 38–41 weeks, birth weight 2.5–4.0 kg (mean 2.9 kg), and Apgar score $\geq$7. Urinary protein and creatinine were estimated using estimation kits base on pyrogallol red and Jaffe's method, respectively. Urinary PCR was calculated by dividing protein concentration (mg/dL) by the creatinine concentration (mg/dL). Urinary protein excretion ranged from 3 to 32 mg/dL (mean 16.24 mg/dL), urinary creatinine ranged 14–65 mg/dL (mean 40.19 mg/dL), and the protein creatinine ratio varied from 0.11 to 0.82 (mean 0.41). The protein creatinine ratio in early neonates of Indian origin ranged from 0.11 to 0.82. The values appeared to decrease with age of infant. A wide scatter was observed compared to older infants, probably due to renal immaturity in early neonates.

P126

Interference of Myeloma Protein in Phosphorous Estimation

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Increased concentration of paraprotein (M-Protein) in multiple myeloma has been reported to result in pseudohyperphosphatemia. This is due to the precipitation of immunoglobulins at the acidic pH of the phosphorous reaction mixture. The analysis in such cases has to be done by minimizing the M-protein effect. We assessed the influence of M-protein in phosphorus estimation using DXC 800 system and compared the results with diluted/deproteinized serum samples. Ten patients with multiple myeloma, 16 polyclonal gammaglobulinemia and 10 healthy individuals were included in the study. Phosphorous estimation was done from neat serum, saline diluted serum and TCA precipitated sample. The phosphorous concentration in all study groups was either low or within normal limits except for one polyclonal gammaglobulinemia patient who had myelodysplatic syndrome. In this patient the phosphorous was 14.1 mg/dl. The variation between the mean phosphorous concentration of neat & diluted serum sample and neat & protein precipitated sample was negligible in healthy controls and polyclonal gamma globulinemia patients and $\sim$2.5 % in the multiple myeloma patients. The M band content in our myeloma patients was 0.4 – 5.3 g/dl and the gamma globulin content was 0.7 – 5.5 g/dl. The gamma globulin in healthy individuals and polyclonal gammaglobulinemia patients was 0.5–1.2 g/dl. Beckman DXC 800 system showed minimal interference in phosphorous estimation upto 5.3 g/dl of M protein and 5.5 g/dl of globulins. However the interference in higher concentrations of M protein needs to be assessed.

P127

Comparison of Serum Sodium and Potassium Levels by Combiline Electrolyte Analyzer and Autoanalyzer

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Electrolytes are necessary for proper cellular functioning and are important in making various clinical decisionsas abnormal values can represent life threatening conditions. It is essential to measure

them accurately. Electrolytes are measured in routine using ion selective electrode which may be by direct or indirect method. The levels of sodium and potassium in serum were analyzed usingcombiline electrolyte analyzer and Roche autoanalyzer and compared statistically. This study was conducted in emergency laboratory of PGIMS Rohtak, where 200 serum samples were analyzed in autoanalyzer (Hitachi Roche) for indirect ISE and electrolyte analyzer (Combiline) for direct ISE for sodium and potassium levels. The mean concentration of sodium was 140.3 ± 7.5 mmol/L using autoanalyzer while 136.6 ± 7.29 mmol/L using electrolyte analyzer ($p < .001$). For potassium mean concentration was 4.1 ± 0.76 mmol/L using autoanalyzer and 3.8 ± 0.72 mmol/L using electrolyte analyzer ($p = .001$). It may be concluded that concluded that there is statistically significant difference between sodium and potassium levels measured by direct and indirect methods so clinician should be aware of two different methods of testing so that properdiagnosiscan be made and different reference range need to be established for the two methods.

P128

A Comparative Study to Evaluate Wash Buffer as an Alternative Diluent to Calibrator S0 in Patients with Very High TSH Concentration

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The analytical range for serum TSH in Beckman Coulter Access2 Immunoassay system is from 0.03 to 100 mIU/L. So, patient samples with severe hypothyroidism often shows very low T3, T4 concentrations and TSH concentration outside the assay range of the analyzer requiring dilution. Our study presents a new method for dilution of serum samples containing serum TSH concentration >100 mIU/L that involves the use of easily available Wash Buffer II solution and hence to eliminate use of expensive Calibrator S0 as recommended diluent. The aim was to evaluate and compare the quality of Wash Buffer II as diluent for TSH assay with that of the conventional Calibrator S0 used as recommended diluent. A total of 50 serum samples were collected where serum TSH concentration >100 mIU/L i.e. outside the assay range requiring dilution. Samples were divided into 2 groups. First group was diluted with Beckman Coulter Access HYPERsensitive hTSH Calibrator S0 in 1:5 dilution. The second group was diluted with Beckman Coulter Wash Buffer II solution in 1:5 dilution. Statistical analysis done and results were compared using Unpaired t-test. The mean TSH concentration for 1st group was found to be 354 ± 34.2 mIU/L whereas for 2nd group it was 349 ± 41.5 mIU/L. This difference is statistically not significant as $p > 0.05$. From the above observation, it can be concluded that Wash Buffer II, which is a routine consumable for Access2 analyzer, can be used as an efficient alternative to Calibrator S0 as sample diluent.

P129

Effect of Matrix on Commonly Measured Analytes in the Emergency Setting Using the Dry Chemistry Technology

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Data on dry chemistry slide technology for serum versus EDTA plasma is unavailable. Hence, this study aimed to evaluate serum vs. EDTA plasma outcomes for selected parameters in 51 healthy individuals using dry chemistry autoanalyzer (*Vitros-350*, *Orthoclinical Diagnostics*). Paired samples were processed simultaneously for Glucose, Urea, Creatinine, Uric acid, TBIL DBIL, AST, ALT, Total protein, Albumin, Cholesterol, dHDL, Triglycerides, Sodium, Chloride, Phosphorus, CPK (total), CPK-MB, LDH, Amylase, Lipase and CRP. Data were analyzed using SPSS-16. Comparable results between serum vs plasma were obtained for Glucose (97.56 vs 99.80, $P = 0.6$), Urea (21.6 vs 20.6, $P = 0.1$), Creatinine (0.74 vs 0.72, $P = 0.5$), DBIL (0.22 vs 0.20, $P = 0.2$), ALT (50.9 vs 51.6, $P = 0.5$), Total protein (7.7 vs 7.8, $P = 0.1$), Cholesterol (163.1 vs 157.1, $P = 0.06$), dHDL(46.9 vs 46.0, $P = 0.2$), Triglyceride (150.2 vs 149, $P = 0.9$), Sodium (141.8 vs 141.3 $P = 0.7$), CPK-MB (15.0 vs 13.7 $P = 0.1$), CRP (0.72 VS 0.64, $P = 0.4$). Statistically significant differences were observed for Uric acid (5.4 vs 5.0, $P = 0.01$), TBIL (0.77 vs 0.65, $P = 0.001$), AST (15.3 vs 3.9, $P < 0.001$), Albumin (4.5 vs 5.0, $P < 0.001$), Chloride (108 vs 105, $P < 0.001$), Phosphorus (3.9 vs 3.6, $P < 0.001$), CPK-total (117.2 vs 43.9, $P < 0.001$), LDH (125.7 vs 92.8, $P < 0.001$), Amylase(75.9 vs 44.8, $P < 0.001$) and Lipase(130 vs 72, $P < 0.001$). This study highlights that parameters like Glucose, Urea, Creatinine, DBil, ALT, Total protein, Sodium, CPK-MB, and CRP which are commonly done in the emergency setting can be reported from EDTA plasma samples also, in cases where serum is not available or lack of time for serum to separate.

P130

Development of In-House IRMA Kit for the Measurement of Serum Thyroglobulin

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Thyroglobulin (Tg), is used as a tumor marker in follow-up of patients with differentiated thyroid cancer (DTC) after thyroidectomy and even for long term management after radioiodine

therapy. At our Centre, we receive ~4800 samples each year for serum-Tg (s-Tg) measurement. Hence, our aim was to develop an in-house Immunoradiometric assay (IRMA) for the estimation of s-Tg in DTC patients who visit for follow-up; as an import substitute for commercial IRMA kits we were using. Standardization of Tg IRMA was carried out using an anti-Tg polyclonal antibody as the capture antibody (raised in camels) and a murine monoclonal antibody labeled with ^{125}I as the tracer. The monoclonal antibody was produced in-house. Since, anti-Tg autoantibodies are known to cause underestimation of Tg in IRMA system, recovery tests were also conducted. The study showed the standardized in-house-Tg assay has a turnaround time of 18 hours. It has a B_{max} of 35–40% and NSB $< 0.2\%$ and was comparable with the commercial kit in respect of assay performance, sensitivity, precision. A very significant ($p < 0.001$) correlation was observed between in-house Tg assay and the commercial kit for Tg values in serum samples (control and DTC patients) with r value of 0.98. Further the in-house assay and commercial assay showed comparable results for % Tg recovery. After satisfactory evaluation the Tg IRMA kit is now in routine use at our Centre. Since 2013, we have produced 570 kits (100 estimatiions) and have analyzed approximately 8500 serum samples.

P131

Reference Intervals and Decision Limits (Ridl) for Serum Lipid Profile in Apparently Healthy Nepalese Adult Population Aged (18–65) Years

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Cardiovascular disease (CVD) is one of the leading causes of morbidity and mortality in the adult population in Nepal. During last decades, the incidences of CVD in Nepal increases drastically throughout the country. Lipid profile tests are the basis for the prediction of CVD. The number of medical laboratory is growing day by day in every part of the country but all most all of the laboratory are using the reference values supplied in the reagents kits which is mainly for either American or European population. RIs are conceptually different for different countries, even for different region in the same countries; at different times, altitude and in same individuals at different ages and condition (eg. preganacy, lactation etc). Therefore, the interpretation of laboratory values with kit values is not the proper way for the prediction of CVD for the Nepalese population. Every lab should have their own reference values for each test in the contest of their locality. For this reason, this study has been designed to establish the reference range for the lipid profile test for healthy adult Nepalese population. Blood samples were collected from the volunteer at different five region of country and sera were separated at the same center and transported to the analyzing laboratory, department of laboratory medicine, Grande International Hospital, Kathmandu, Nepal. Total 630 apparently healthy individual were enrolled in this study. Lipid profile (Total cholesterol, triglyceride, HDL-Cholesterol) were analyzed by using vitros reagent on Vitros 250 instrument. LDL-Cholesterol was calculated using Friedewald equation. Reference materials and panel sera were used for the standardization of test results. By using secondary exclusion criteria - the latent abnormal values exclusion (LAVE), reference intervals (RIs) were derived by both parametric and non-parametric method by using Reference Master and StatFlex software. But non parametric methodology for determination of RI was adopted as most of the biochemical parameters included revealed non Gaussian distribution. The reference interval (RI) values (median and range in mg/dl)-upper limit) for total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol obtained were 147(98–215), 117(56–363), 38(23–62) and 80(39–137), respectively. However, gender wise analysis demonstrated higher range for Cholesterol, triglyceride and LDL-cholesterol in men than female while HDL-cholesterol has higher range in female than male. It can be suggested that the reference interval values for lipid profile obtained by this study can be used as reference Intervals for the interpretation of laboratory values in the diagnosis, care and treatment of cardiovascular disease patients in context of Nepalese population.

P132a

Vitamin D Deficiency by LC-MS/MS in Indian Population

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Vitamin D can influence 300 genes of our body. Vitamin D receptors are present in various organs and tissue. Several studies have demonstrated low serum Vitamin 25 (OH) D levels in population across India. In North India, 96% of neonates, 91% of healthy school girls, 78% of healthy hospital staff and 84% of pregnant women are found to be deficient in Vitamin D. The criteria used for defining hypovitaminosis D in most of studies was a serum 25(OH)D level below 50 nmol/L. Vitamin D deficiency has strong association with flu, anemia, cancer, diabetes, hypertension, cardiovascular risks, brain damage, paralysis, TB, asthma, infertility, chronic Kidneydisease, autoImuune disorders, numbness, convulsions, weak bones, back pain, unexplained muscle pain, fatigue. Maternal deficiency of Vitamin D is linked with abnormal fetal growth and gestational diabetes. Study of Khadgawat etal (JAPI Sept 2010 vol 58; p539–542) shows very high prevalence (96.7%) of vitamin D deficiency in Asian Indian patients with fragility hip fracture. Psychitric patients presenting with their first episode of psychosis are more likely to have vitamin D deficiency than their healthy peers (Schizophr res 2013;150:533–537. To get a true reading of Vitamin D level, technique should be used that can detect both 25 (OH) D2 & D3 equally. Vitamin D is produced in two forms-Vitamin D2 & Vitamin D3, which differ by the presence of a double bond and methyl group on the aliphatic side chain. Total 25 (OH) D should represent the total amount of Vitamin 25 (OH) D (Both D2 &D3) that is circulating. Present common immunoassay method of measuring 25 (OH) D, has drawbacks for these assays can measure only VitaminD3, as binding protein shows a higher affinity for Vitamin D3 than Vitamin D2. LC-MS/MS is currently the best technique for the correct quantification of 25OHD3 &25OHD2 and has also the capability to overcome most of problems associated with protein binding assays. We studied Vitamin D levels in 3520 subjects by LC-MS/MS technique by chromatographically seprating interferences due to epimers. Epimers are non-super imposable (or non mirror image) that differ only in the configuration of one carbon atom. Epimers are compounds with the same molecular weight as Vitamin D metabolites and from

the same mass to charge parent and product ion pairs upon ionization. Sepration of epimers is essential to avoid false estimate of Vitamin D. Results will be presented.

P132b

Comparison of Results of Biochemistry Analytes by Two Different Commercially Available Reagents on a Conventional Laboratory Analyzer at an Oncology Centre

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Consistent and reproducible results from different measurement procedures are a must for diagnosis and appropriate treatment of diseases. The objectives of the study were to compare the results of the biochemistry analytes processed by two different commercially available reagents on a conventional laboratory analyser and to calculate total error for the analytes subjected to comparison. This study was designed for comparing results of three analytes, Creatinine Kinase (CK), CK-MB and Lactate by using reagents from Randox laboratories (India) Pvt Ltd. and from Beckman Coulter India Pvt. Ltd. It also included determination of within-run (N = 20) and between-run imprecision (N = 20), inaccuracy (N = 20) and method comparison by both the reagents on Beckman Coulter AU 2700 (N = 40). Total error was calculated for validation of the analytical process. The results indicate that within-run imprecision CVs were all below 5.5 % for both the reagents. Between run CVs for all analytes were below 5%. All the analytes have fulfilled requirements for inaccuracy and total error as per the quality specifications. We conclude that t69he reagents from both the manufacturers had low CV values and had satisfactory accuracy and precision and are extremely stable. Method comparison study showed excellent correlation between both the reagents. Hence, the reagents from the two different manufacturer's can be used for routine testing of the respective analytes.

P133

Evaluation of Serum Levels of Adiponectin as Biomarker for Diagnosis of Ovarian Cancer

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Current strategies for detection of epithelial ovarian cancer (sixth most common cancer) are based on biochemical markers like Carbohydrate Antigen 125 (CA125) and imaging techniques, which have low sensitivity and specificity. Many proteins including Adiponectin are being evaluated as screening markers for detection of ovarian cancer has been evaluated in this study. This hospital based case control study was conducted in the Departments of Biochemistry in collaboration with Obstetrics and Gynecology, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, including 30 malignant ovarian cancer patients, 30 benign ovarian tumors and 30 healthy controls were enrolled with their consent. After detailed history and clinical evaluation, blood samples were drawn for estimation of various biochemical parameters namely fasting plasma glucose, serum LFT, KFT, Lipid Profile, Insulin, CA-125 and Adiponectin by standard methods. Mean age of healthy controls, benign ovarian and malignant ovarian cancers were 48.5, 43.6 and 50.1 years respectively. The median of serum CA-125 levels in healthy controls - 12.6 u/ml, in benign ovarian conditions - 209.6 u/ml and in malignant ovarian conditions - 1619.6 u/ml. Using Kruskal Wallis test the levels were statistically significant (<0.001). The median of S. adiponectin in healthy controls was 13.6 μg/ml, in benign ovarian conditions it was 8.0 μg/ml and in malignant ovarian conditions the median was 5.1 μg/ml. Using Kruskal Wallis test and groups were found to be significantly different (<0.001). This study provides evidence that serum levels of CA-125 were increased in ovarian cancers. The levels of adiponectin in malignant groups were significantly low as compared to benign groups and healthy controls.

P134

Evaluation of Effects of Metformin in p53 mutant Epithelial Ovarian Cancer cells

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Epithelial ovarian cancer is one of the most lethal gynecological malignancies. Despite the progress in surgical and therapeutic strategies, resistance to chemotherapy is still a major concern. Chemotherapeutic agents cause cytotoxicity primarily by the induction of apoptosis and p53 status is a key factor in determining the efficacy of apoptotic signalling. p53 is the most commonly mutated in ovarian cancer hence we aimed to study role of metformin (an antidiabetic drug) in p53 mutated cancer cell line since it has shown putative effects in many solid tumors. SKOV-3 and OAW42 ovarian cancer cell line was used. The cancer cells were treated with metformin. MTT, Flow cytometry and Western blotting were used to characterize the effects of the different treatments. Treatment with metformin resulted in an increase in the number of cells arrested in the G0/G1, S and G2/M phase of the cell cycle in SKOV3 and OAW42 respectively. Moreover there was upregulation of Bax and downregulation of Bcl-2 protein and increased apoptosis in SKOV3 and OAW42 ovarian cancer cells. These findings support the potential of metformin to be used as chemo-adjuvant. Moreover it reflects its ability to sensitize cancer cells to apoptosis independent of p53 status.

P135

Studying of the Association between EGR1 and CR1 in Specific Cell Lines *In Vitro*

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EGR1, early growth response protein 1, is an early response transcription factor which has tumor suppressor activity. Cripto-1 is a regulatory gene involved in embryogenesis and promotes carcinogenesis. It would be interesting to identify if the expression of these two genes are inter-linked through a specific pathway. Glioblastoma (U87) cell line was cultured *in vitro*. Based on MTT, U87 cells were treated with 10 μM of Erlotinib for 24 hours and expression of Egr1 and Cripto1 were studied. To further validate the regulation of expression, the cDNA was amplified for Egr1 and Cripto1 and the product ligated to 1013 vector. After transfection, stable expression was obtained. PCR of untreated and treated cells show a simultaneous change in both EGR1 and CR-1 expression on treatment with Erlotinib. β-actin was used as internal control. 1750 bp specific Egr1 band and 679 bp specific band for Cripto1 was aligned completely to human gene reference sequence in NCBI database. Increased expression of human Egr1 and Cripto1 were observed in transfected cells and modulation of gene expression was validated using RT qPCR. Initial results indicate an inverse correlation between EGR1 and CR-1 expression patterns. The dose-response relationship, exact pathways involved and the dynamicity of this connectivity is being established. Signals from various growth factors cascade through cardinal signalling PI3 K/Akt and MAPK. We are currently studying the molecular cross-talk between these pathways and their regulatory role in controlling the action of EGR1, CR-1 and their link with genesis and modulation of cancer.

P136

Association of Thyroid and Parathyroid Hormones with the Severity of Benign Prostatic Hyperplasia

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Benign Prostatic hyperplasia (BPH) is a multifactorial disease. Thyroid and Parathyroid hormones are known to stimulate prostate growth and are implicated in prostate carcinogenesis. The present study was designed to assess the levels of thyroid and parathyroid hormones and their association with prostate size in BPH patients. 40 BPH cases and 40 controls were enrolled in the study. Thyroid and parathyroid hormones were estimated in both the groups. Free T3 and free T4 were significantly increased and TSH was significantly reduced in BPH cases when compared with controls. Free T3 ($r = 0.341$, $p = 0.031$), TSH ($r = -0.431$, $p = 0.005$) and parathyroid hormone ($r = 0.353$, $p = 0.026$) were significantly associated with prostate size in BPH cases. Stepwise regression analysis showed that free T3 act as predictor of prostate size in BPH ($R^2 = 0.175$, $\beta = 0.329$, $p = 0.038$). Also free T3 was found to be significantly increased in BPH cases with higher prostate size ($p = 0.046$). We conclude that thyroid hormones are elevated in BPH cases and in particular free T3 is associated with severity of BPH. PTH was neither elevated nor associated with severity of the disease.

P137

Comparison of Pre and Post Radiotherapy Serum Butyrylcholinesterase Levels in Oral Cancer

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Oral squamous cell carcinoma (OSCC) is one of the most common malignancies recognized nowadays, and represents a public health problem. The clinical and histological features alone cannot always accurately predict whether potentially malignant disorders of the oral mucosa remain stable, regress or progress to malignancy. Identification of molecular markers which can predict disease progression is necessary for better management of these disorders. Studies have shown correlation of butyrylcholinesterase with tumorigenesis, cell proliferation and cell differentiation. So, we sought to estimate and compare serum butyrylcholinesterase levels among healthy controls and biopsy proven oral cancer patients before and after radiotherapy. Institutional Ethics Committee gave us the permission conduct this study. After obtaining consent from biopsy proven oral cancer patients ($n = 39$) 2 ml of blood was taken twice once before onset of any definitive treatment and again one day after completion of radiotherapy. Simultaneously, same amount of blood was taken form age and sex matched healthy controls ($n = 20$). After clot formation samples were centrifuged and serum was collected for estimation of butyrylcholinesterase. Median values of pre-treatment serum butyrylcholinesterase levels were significantly elevated ($p \leq 0.0001$) in oral cancer patients [6956 IU/l] as compared to that of controls [1725.5 IU/L]. There was a significant increase in median values of pre-treatment serum BChE levels with advancement of oral cancer. The median values of post treatment BChE levels of cancer patients in different stages were significantly decreased as compared to their respective pre-treatment levels. Thus, there could be a role for butyrylcholinesterase in the management of oral cancer.

P138

Metformin Induces Modulation of Differentiation Markers in Colorectal Cancer Cell Lines

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Metformin is an anti-diabetic drug that has anti-carcinogenic activity. Its anticancer activity may be attributable to its ability to induce differentiation in cancer stem cells (CSCs). CSCs are

resistant to conventional chemotherapy. We wanted to investigate if inducing differentiation can help target CSCs. Colon cancer cell lines HT29 and HCT116 were cultured *in vitro.* CSCs were evaluated by flow cytometry (CD44, CD166). MTT assay helped establish maximum tolerable non-toxic dose for Metformin. Metformin-induced apoptosis was evaluated using flow cytometry. HT29 and HCT116 cells were treated with Metformin for 2 weeks. Whether differentiation was induced, was analyzed by evaluating CDX1 (transcription factor) expression using RT-PCR and appearance of Cytokeratin 20 (CK20), a positive marker of differentiation by flow cytometry. HT29 (moderately differentiated) contained a much lower percentage of CSCs (8%) compared to HCT116 (24%) which are poorly differentiated. After metformin treatment for two weeks, expression of CK20 and CDX1 was found to be altered by flow cytometry and RT-PCR respectively. Our findings indicate that HCT116 and HT29 cells are attributed to be more and less aggressive respectively by virtue of presence of different population of CSCs. As CSCs are associated with difference in differentiation patterns, doses of metformin tolerated by HT29 and HCT116 also differ. This reflects the difference in resistant and undifferentiated CSC population in each cell line. Initial findings indicate that metformin may induce differentiation in the undifferentiated CSC present in colorectal cancer cell lines HT29 and HCT116, thereby indicating its potential therapeutic role in targeting resistant CSCs.

P139

Effect of Flavonoids on Reversal of TGF- β -Induced Epithelial to Mesenchymal Transition in Prostate Cancer (PC-3) Cell Line

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Transforming growth factor-β (TGF-β) induces EMT in epithelial cells and is necessary for acquisition of invasive phenotype in Prostate cancer. It has been hypothesized that prostate cells adopt embryonic signalling pathways (such as Wnt) that are generally silent. The purpose of this study was to evaluate the effect of flavonoid Quercetin on TGF-β-induced EMT in Prostate cancer (PC-3) cell line. To evaluate the effect of flavonoid Quercetin on the reversal of TGF-β-induced EMT in Prostate cancer (PC-3) cell line. In this study, we induced EMT in Prostate cancer PC-3 cells by treating them with TGF-β and subsequently studied the effect of Quercetin on reversal of EMT. Expression of epithelial and mesenchymal markers was screened by real-time PCR before and after treatment. The expression of epithelial markers was found to be higher in control untreated cells as compared to cells treated with TGF-β. However the expression of mesenchymal markers was found to be lower in control untreated cells and was up-regulated in induced state. Treatment with Quercetin prevented TGF-β-induced expression of N-cadherin and Vimentin and increased the expression of E-cadherin in PC-3 cells. The relative down-regulation of transcription factors Snail, Slug and Twist upon Quercetin treatment further confirmed that Quercetin decreased TGF-β-induced EMT. Quercetin, a plant flavonoid, prevented TGF-β induced migration of PC-3 cells. Quercetin may prevent cancer metastasis by regulating the components of Wnt Pathway.

P140

Study of Serum Lactate Dehydrogenase as a Prognostic Tool for Non-Hodgkin's Lymphoma

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Serum Lactate Dehydrogenase (LDH) level is an established marker of intermediate and high grade Non-Hodgkin's Lymphoma (NHL). We wanted to study it as a prognostic tool in all stages and grades of NHL patients. We determine serum LDH level in Non-Hodgkin's lymphoma cases during different stages of disease and its prognostic significance in Non-Hodgkin's lymphoma. This retrospective study was conducted on 30 diagnosed cases of NHL attending the Radiotherapy OPD of S. S. G. Hospital and Medical College Baroda, Vadodara. Serum LDH levels were measured before treatment, during treatment and in follow-up, on fully automated biochemistry analyser Cobas C-311. LDH levels were significantly higher in patients with high grade NHL that decreased after treatment and an increase in serum levels of LDH was found in cases that showed relapse of disease. From the current study we conclude that serum LDH levels are very informative in assessing the prognosis of NHL. In current study, sample size was small and also some patients were lost to follow-up. Further study with more number of cases will be more conclusive.

P141

Bacterial Sepsis in Cancer Patients: Key Role of CRP and White Cell Count

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Bacterial infection is a frequent complication in cancer patients. C-reactive protein (CRP) is used mainly as a marker of inflammation and neutrophilia is an indicator of bacterial infection. In this retrospective study we have tried to determine the significance of these parameters in predicting bacterial sepsis in cancer patients. During a period of 6 months, 146 samples (Blood-PICC/peripheral blood/Hickman Catheter, stool, urine, sputum, fluids, and swab) were received for suspected infection from hemato-lymphoid and hematopoietic stem cell transplant (HSCT) units. Serum CRP and Neutrophil values of these samples were estimated as part of routine investigations as per institutional protocol. Statistical evaluation was carried out to assess significance of CRP and neutrophil count in patients with culture positive samples. Of 146 samples studied, 69 showed bacterial growth. Of these 67% (33/49) samples showed leucopenia, 33% (16/49) samples were non-leucopenic. Amongst the leucopenic samples, 79% (26/33) showed CRP values >5 mg/dl and 31% (5/16) samples from non-leucopenic cases had CRP value >5 mg/dl ($p = 0.0019$). In leucopenic cases neutrophilia was

observed in 18% (6/33) cases, while 50% (8/16) of non-leucopenic cases showed neutrophilia ($p = 0.0483$). Although Serum CRP levels are a non-specific indicator of inflammation, a value of more than 5 mg/dl is a reliable indicator for suspected bacterial infection in leucopenic patients; however the relationship between the neutrophil counts and clinical sepsis was not clear in our group of patients.

P142

Circulatory Micrornas in Prostate Cancer Progression

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Circulatory MicroRNAs (miRNAs) are a new class of small multifunctional non-coding RNA molecules that regulate fundamental cellular and developmental processes by regulating the stability or translational efficiency of targeted messenger RNA. Prostate Cancer (PCa), the second most common non-cutaneous malignancy in men worldwide still lacks in terms of a sensitive and specific biomarker. Circulatory miRNA- expression profiling of Prostate cancer cells in serum, urine and plasma has led to the identification of signature miRNA associated with PCa diagnosis, staging, progression, prognosis and response to treatment. This review aims to highlight the recent findings of miRNAs involved in PCa progression as well the functional consequences of miRNA dysregulation in various regulatory pathways of PCa. A thorough literature survey was carried out to prepare a comprehensive list of circulatory miRNAs differentially expressed in PCa cells as compared to normal Prostate cells. This was followed by identification of their downregulated genes and pathway enrichment analysis using online tools and databases. Both tumor suppressor activity and tumorigenic properties have been assigned to specific miRNAs such as miRs-100, 125b, 141, 143, 200b, 195, Let 7i, 181a-2, 24, 26b, 30c which are being expressed virtually at all relevant stages of Prostate cancer progression, including apoptosis, resistance, tumor cell proliferation, migration, invasiveness, angiogenesis and metastasis. The investigation of the myriad of circulatory miRNAs expression would enhance the understanding of their role as potential biomarkers of PCa and also their downstream target genes that might represent the activated oncogenic pathways or target protein genes involved in PCa.

P143

Estimation of Mitochondrial DNA Copy Number as a Prognostic Marker in Childhood Acute Lymphoblastic Leukemia (ALL)

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Quantitative changes in mitochondrial DNA (mtDNA) have been identified in various tumors and have been evaluated for prognostic significance. Studies in pediatric ALL have shown conflicting results about copy number changes but the mtDNA copy number in childhood ALL patients could be of possible prognostic significance and/ or help in predicting treatment outcomes. We study mtDNA copy number in childhood ALL, before and after induction and to correlate the mtDNA copy number with clinico-pathological variables in patients. Bone marrow aspirates from 32 pediatric ALL subjects were obtained after informed consent. Lymphocyte isolation by ficoll-hypaque, followed by total cellular DNA extraction and real-time PCR using mitochondrial genome specific primers was done to estimate copy number at baseline and after induction phase of chemotherapy (4 weeks), normalized to beta-actin gene. The copy number was correlated with age, lymphocyte count and cytogenetic parameters and compared in patients and controls. The mean age of subjects was 9.32 years. The median mtDNA copy number (mt copies/ beta-actin copies) was 107.8 at baseline and 57.18 after induction (p value $= 0.0412$). There was significant difference in copy numbers between patients and controls (p value $= 0.0024$). There were no statistically significant correlations with age, leukocyte count and cytogenetic parameters. The mtDNA copy number showed a significant decrease after chemotherapy. Analysis of the underlying mechanistic aspects in a bigger sample set may provide better insight into the prognostic significance, if any of mtDNA copy number in childhood ALL.

P144

To Evaluate the Role of IGF-1 Gene Expression in CML Disease Progression

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Chronic myeloid leukemia (CML) is a clonal disorder characterized by Philadelphia (Ph) chromosome, resulting from reciprocal chromosomal translocation t(9;22)(q34; q11). CML typically evolves from a chronic phase (CP), through a brief period of accelerated phase (AP), and ends up in more aggressive blast crisis (BC). The Insulin-like Growth Factor (IGF) family of proteins are known to play an important role in regulating cell proliferation, differentiation and apoptosis and may be important in promoting carcinogenesis. We characterized IGF-1 gene expression in 100 CML patients in different clinical phases (CP, AP and BC) and 100 healthy controls by SYBR Green based qRT-PCR. We also studied IGF-1 expression in K562 CML cell line with respect to a non-leukemic cell line (HBL 100). IGF-1 expression was expressed as fold change ($2^{-\Delta\Delta ct}$ method). We observed a 5.7 fold increase in IGF-1 gene expression in CP cases, whereas the increase in AP, BC cases was 7.3 fold, which was statistically significant ($p = 0.02$). Patients with major molecular response to Imatinib, and those with loss of molecular response had 2.94 and 3.19 fold increase in IGF-1 expression respectively ($p = 0.5$). Also, there was no significant correlation of IGF-1 expression and haematological response to Imatinib ($p = 0.25$). In K562 CML cell line IGF-1 gene expression was up-regulated 8 fold in comparison to the HBL 100 non-leukemic cell line. It may be concluded that IGF-1 gene expression increases progressively in CML with advancement of disease.

P145

Association of Dyslipidemia, Insulin Resistance and Prostate Specific Antigen with Prostate Cancer

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The incidence of Prostate Cancer is increasing day by day all over the world. At present the incidence is about 5 per 1, 00,000 in India. This study is designed to associate dyslipidemia, insulin resistance and prostate specific antigen (PSA) with onset of prostate cancer. In this study Body mass index (BMI), lipid profile, serum glucose, insulin and insulin resistance and PSA were estimated. We study the effects of dyslipidemia, altered blood glucose, Insulin Resistance and PSA as risk factors for development of prostate cancer. The study was conducted total 150 in which 75 patients of prostate cancer (case) and 75 healthy individuals as control. BMI was calculated as "weight in kilograms divided by height in meters squared (kg/m^2)", and 5 ml blood samples were drawn to determine fasting blood glucose, serum lipid profile, serum insulin and PSA. Blood glucose, total cholesterol, HDL cholesterol, and triglyceride, were measured by using enzymatic kits of auto analyzer. VLDL-cholesterol and LDL cholesterol were calculated by Friedwald's Formula. Serum insulin and serum PSA were estimated by immune-enzymatic assay. Insulin resistance was assessed by, "Homeostasis Model Assessment Insulin Resistance Index" (HOMA-IR) and calculated as: "fasting glucose (mg/dL) × fasting insulin (mU/mL)/405". Clinical variables such as age, BMI, lipid profile, insulin resistance and PSA in case and control groups were compared using the Unpaired Student's t-test. We found that the level of glucose, insulin, insulin resistance, PSA and BMI were significantly increased in prostate cancer patients as compared to control. In prostate cancer patients HDL cholesterol significantly decreased ($P < 0.001$), while Total Cholesterol, TG, LDL, VLDL were significantly increased ($P < 0.001$) as compared to control group. This study has shown significant association of high BMI, dyslipidemia, insulin resistance and PSA with prostate cancer.

P146

To evaluate the Effects of a Polyphenolic Flavonoid Quercetin on the Metastatic Characteristics of SK-MEL-28 Human Melanoma Cells

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Paucity of effective treatments in melanoma prognosis applauds current neoplasia studies to understand the genetic and molecular insights of tumor's repopulation by identifying factors involved in Epithelial to Mesenchymal Transition (EMT) which triggers metastasis. Quercetin a majorly found flavonoid in nature is known to ablate tumor progression by reversing EMT. We study the effect of Quercetin on the EMT induced in melanoma cell line. Human SK-MEL-28 melanoma cells were treated with Quercetin post their culture on plastic and collagen coated surfaces. Morphological changes, proliferation rate, migratory and chemotactic property variations of cells due to collagen before and after treatment were analyzed. Variations in EMT markers were also analyzed using Real Time PCR. Quercetin treated cells on collagen matrix showed reduced proliferation as compared to cells on plastic matrix. Because of collagen's chemotactic invasion effect, the cells showed faster migration as compared to on plastic matrix and after treatment this migration rate was decreased. Quercetin treatment also led to decrease in expression of N-cadherin, VCAM-1 and Vimentin and increase in the expression of E-cadherin, MMP-9 and Cyclin-D1 which strengthens its role in EMT attenuation in melanoma. Our results define Quercetin's effectiveness in reducing the proliferation and migratory capacity of melanoma cells thus headlining its prospects as a new therapeutic drug for melanoma treatment.

P147

Role of Fluoride in Osteosarcoma

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There are conflicting reports about the carcinogenic potential of fluoride especially in osteosarcoma. The present study was planned to analyse serum fluoride levels in osteosarcoma. Serum fluoride levels were studied in 75 subjects. They were categorised as patients with osteosarcoma (group III, $n = 25$), age- and sex-matched subjects with bone-forming tumours other than osteosarcoma (group II, $n = 25$) and musculo-skeletal pain (controls, group I, $n = 25$). Fluoride levels were analysed in serum by ISE method. Mean serum fluoride concentrations were significantly higher in patients with osteosarcoma as compared to the other two groups. The mean value of fluoride in patients with other bone forming tumors was approximately 50% of the group of osteosarcoma; however, it was significantly higher when compared with patients of group I. Alkaline phosphatase levels were significantly raised in osteosarcoma patients as compared to group I and non-significant difference as compared to group II. The findings of higher serum fluoride levels in osteosarcoma patients as compared to controls suggest a role of fluoride in the disease. Further research, such as large case-control studies are recommended.

P148

Interleukin-8-251A/T and Interleukin-10-1082A/G Gene Polymorphisms in North Indian Patients with Colorectal Cancer

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Interleukin-8 is an important chemokine for regulation of inflammatory response. It may be involved in development and progression of many human malignancies including colorectal cancer (CRC). Interleukin (IL)-10 is major anti-inflammatory cytokine that plays crucial role in regulation of immune system and its activity may

be important for clinical outcome of CRC. We investigate allele and genotype frequencies and associated risk of IL-8 -251A/T and IL-10 -1082 A/G polymorphism on CRC susceptibility risk. For this study, peripheral blood samples of 65 healthy controls and 43 clinically and histo-pathologically confirmed CRC patients were obtained. DNA was extracted from peripheral blood and genotypes were analyzed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Out of 43 CRC patients, 29 were males while in controls 33/65. Mean age of patients was 51.3 ± 14.6 years and 48.2 ± 13.9 years in controls. It was observed that genotype frequencies of IL-10 variants AA and GA were significantly higher ($p < 0.05$) in CRC patients as compared to controls. However, IL-8 genotype frequencies were not significant as compared to controls. Genotype frequencies of IL-8 -251A/T were TT 39.5%; AT 46.5% and AA 13.9%. Moreover, genotype frequencies of IL-10 -1082A/G were AA 83.7%, GA 16.3% and GG 0%. The frequency of mutant G allele (IL-10) was 91.8% in CRC patients vs. 99.2% in controls ($p < 0.05$). This study shows that Variant allele and genotype of IL-10 (G/A) was significantly associated with CRC susceptibility risk. However, there was no association of IL-8 -251A/T gene polymorphism with susceptibility to CRC risk in Indian patients.

P149

Elucidating the Link Between Osteoblastic Differentiation of Human Bone Marrow Derived Mesenchymal Stem Cells and Tumorigenesis

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Human Mesenchymal Stem Cells (MSCs) are multipotent cells that have application in treatment of degenerative bone diseases. They have the potential to differentiate into bone, cartilage and other tissues. However, malignant transformation of MSCs has been reported and is a stumbling block. We elucidate the relationship between osteogenic differentiation of MSCs and tumorigenesis. MSCs were isolated from human bone marrow and grown in DMEM-Low glucose with 15%FBS, Stempro SFM for MSCs at 37°C and 5% CO_2. MSCs in 3^{rd} – 5^{th} passage were differentiated into osteoblasts by adding specific inducers like beta glycerol-phosphate, ascorbic acid, Dexamethasone with and without BMP-2. Flow cytometry was used to characterize MSCs (CD90, CD105) and differentiated osteoblasts (Osteopontin, Osteocalcin). qPCR was performed to check the mRNA expression of PCNA and Ki-67 in MSCs and osteoblasts. Morphology, HandE and Alizarin staining demonstrated MSCs and osteoblasts. Flow cytometry confirmed presence of MSCs and osteoblasts by specific markers. MSCs differentiated into osteoblasts in 3 weeks with an efficiency of 50 ± 11.1% (without BMP-2) and 82.6 ± 3.24 (with BMP-2). Decrease in mRNA expression of PCNA and Ki-67 was observed as MSCs differentiated into osteoblasts. Flow cytometry confirmed purity of MSCs (93–98%) isolated from human bone marrow by presence of characteristic cell surface markers. Appearance and quantification of bone matrix proteins *viz.* Osteopontin and Osteocalcin confirmed osteoblast formation. Decrease in mRNA expression of Ki-67 and PCNA is an interesting phenomenon observed during osteogenic differentiation and gives an insight into the possible association between differentiation and tumorigenesis.

P150

Inflammatory Promoter Genetic Variations (Intrinsic Factors) with Tobacco Attributes (Extrinsic Factors) May Generate Susceptibility Risks and Severity of Prostate Carcinoma

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IL-18 is a pro-inflammatory cytokine expressed on various cells including prostate gland elements, and is a crucial mediator of immune responses with anti-cancerous properties. IL-10 is an anti-inflammatory cytokine that is linked with tumour malignancy which causes immune escape. The pin-pointed objective is that IL-18 (pro-) and IL-10 (anti-) inflammatory genetic variants at -607 C/A-137G/C and -819C/T, -592C/A, respectively, may generate susceptibility and severity risk with various modes of tobacco exposure in prostate carcinoma (PCa) patients. The present study was conducted with 540 subjects, comprising 269 prostate carcinoma patients and 271 controls. Genotyping was performed by PCR-RFLP and confirmed by real time PCR probe-based methods. The findings indicated that the mutant heterozygous and homozygous genotype CC and GC + CC showed significant negative associations ($p = 0.01$, OR = 0.21; 95% CI: 0.08–0.51 and $p = 0.011$, OR = 0.43; 95% CI: 0.22–0.81, respectively) thus, less chance to be diagnosed as cancer against GG genotype of tobacco smoking patients. In addition, a heterozygous GC genotype at the same locus of IL-18 pro-inflammatory cytokine may aggravate the severity (OR = 2.82; 95%CI 1.09–7.29: $p = 001$) so that patients are more likely to be diagnosed in advanced stage than with the GG wild homozygous genotype. Our results also illustrated that anti-inflammatory cytokine (IL-10) genetic variants, have profound effects on severity of the disease, as -819 TC (OR = 4.60; 95%CI 1.35–15.73), and -592 AC (OR = 5.04; 95%CI 1.08–25.43) of IL-10 in tobacco chewers and combined users (both chewers and smokers) respectively, are linked with diagnosis in more severe stage than with other variants. We conclude that promoter genetic variants of IL-18 and IL-10 with various modes of tobacco exposure may influence not only susceptibility risk but also severity in prostate cancer.

P151

Comparing Mechanical Stiffness, EMT/MET and Stemness in Colon Carcinoma Cell Lines and Human Bone Marrow Derived Mesenchymal Stem Cells

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Epithelial to mesenchymal transition (EMT) and mesenchymal to epithelial transition (MET) are two biological processes that are known to play an important role in tumor invasion and metastasis. Mesenchymal stem cells (MSCs) are endowed with migratory properties that help them in homing in to tumors. Mechanical stiffness of cells is also a physical property associated with their migratory capacity. We wanted to study migratory and stem cell properties of metastatic colon cancer cells and compare them with MSCs. Colon cancer cells (HT29 and HCT116) and human bone marrow derived MSCs were cultured *in vitro*. EMT/MET and stem cell markers were evaluated for colon cancer cells with varying degrees of metastatic potential and compared with that of MSCs by RT-PCR. The mechanical stiffness of cellular surfaces of the different groups of cells (HT29, HCT116 and MSCs) was studied by atomic force microscopy with the help of colloidal tips. mRNA expression of EMT/MET markers and stem cell properties (β-integrin, ABCG2) were observed in HT29 (moderately differentiated and less metastatic), HCT116 (poorly differentiated and more metastatic) and MSCs (bone marrow derived multipotent stem cells). Mechanical properties of the three different types of cells were evaluated using atomic force microscopy. Differences in expression patterns of EMT/MET markers, stemness markers (β-integrin, ABCG2) and mechanical (elastic) properties of HT29 (less metastatic), HCT116 (more metastatic) and MSCs reflect basic differences between these different cell types. Our findings also imply that mechanical properties of cells may influence their migratory capacities or vice versa.

P152

Coenzyme Q10 and its Emerging Role as an Antineoplastic Agent

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Coenzyme Q10 (CoQ10) is a naturally occurring, lipid soluble substance. It is a key molecule in all energy requiring processes including proliferation, apoptosis, angiogenesis and immune function, suggesting the potential for multiple roles in the initiation and progression of cancer. It is also known as ubiquinone. It is an antioxidant and a redox coenzyme of the electron transport chain. This literature review was performed by conducting a systematic search of PUBMED, MEDLINE and PMC, including all articles up to November 2014. All articles were reviewed and were included if they were relevant to the topic, and deemed to be of good quality. The references for each article were reviewed to identify further articles of relevance. CoQ10 levels are altered in a number of oncological as well as non-oncological diseases. CoQ10 has an impact on the expression of many genes involved in metabolism, cellular transport, transcription control, and cell signalling, making CoQ10 a potent gene regulator. Supplementation of Co Q10 with chemotherapy to cancer patients reduces the tumour marker level thereby offering better cancer prognosis by reducing the risk of developing cancer recurrence and metastasis, improved quality of life. CoQ10 therapy has no serious side effects in humans and new formulations have been developed that increase CoQ10 absorption and tissue distribution. CoQ10 has a role in carcinoma breast, cervix, lung, prostate, melanoma, cancer chemotherapy and cancer related fatigue. Future trends involving CoQ10 in many cancers needs more clinical trials for better understanding of CoQ10 efficacy.

P153

Serum Protein Fractions, Renal Markers, and Electrolytes in Multiple Myeloma

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Multiple myeloma (MM) is a malignant proliferation of monoclonal plasma cells leading to clinical features that include hypercalcaemia, renal dysfunction, anaemia, and bone disease (CRAB features). Renal involvement and renal failure is one of the major complications of MM along with bone features and anemia. The study was conducted to evaluate serum protein fractions in MM and an attempt was also made to correlate these fractions with renal markers, electrolytes, and haemoglobin (Hb). The data for serum protein fractions, renal markers, electrolytes, and Hb were retrospectively collected from the laboratory records of 25 MM patients who attended PIMS and RC. The age group of the patients ranged from 49–86 with a mean age of 67.5 years. The study comprised of 14 males and 11 females. The mean values of total protein, albumin, alpha 1, alpha 2, beta and gamma fractions were found with total protein, alpha 2 and gamma increased while albumin decreased than the biological reference range. Creatinine values increased while sodium and haemoglobin values decreased compared to biological reference range. Alpha 2 showed a significant positive correlation with creatinine ($p < 0.024$) while significant negative correlations with sodium ($p < 0.03$) and Hb ($p < 0.043$). Total protein showed significant negative correlations with sodium ($p < 0.048$) and Hb ($p < 0.01$) while gamma showed a significant negative correlation with Hb ($p < 0.007$). Alpha 2 showed significant correlations with creatinine, sodium and Hb; total protein showed significant correlations with sodium and Hb while gamma had only with Hb.

P154

Atypical Presentation of 'M' Protein on Protein Electrophoresis: A Case with Multiple Myeloma

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Multiple myeloma is a malignant disease of plasma cells that manifests as disease in the bone marrow. Monoclonal protein in the blood and /or urine along with evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder. Agarose gel electrophoresis is a method for detecting monoclonal protein. The 'M' protein usually migrates as a single entity (localized band) peak in gamma or beta globulin region and very rarely in alpha-2 globulin. In the following case, we present a rare occurrence of M – Spike in the alpha -2 region of Serum protein electrophoresis. A seventy –five year old man was admitted to Medanta- The Medicity, Gurgaon, India with complaints of anemia, hypertension and shortness of breath. He was shifted to Heart Command Centre in view of Hypertension and shortness of breath and advised for CAG. Serum protein electrophoresis and Immuno-fixation electrophoresis were performed on Hydrasys 2 (Agarose Gel Electrophoresis). Serum Protein Electrophoresis was performed and the results showed decreased albumin (48.7% with a

reference range of 59.8–72.4,) or 2.63 g/dl with a reference range of 3.50–5.78 g/dl and increased alpha-2 of 1.56 g/dl (reference range: 0.31–0.87 g/dl). The M-spike was suspected in alpha-2 region on the electrophoresis results and hence Immuno-fixation was performed for further evaluation. Immuno-fixation electrophoresis further confirmed of Monoclonal Gammopathy in alpha-2 region consisting of Lambda. The patient was advised for IgD/IgE. The Free Light Chain assay reported free kappa, free lambda as 18.02 mg/l and >1650 mg/l respectively. The bone marrow is cellular and shows ~51.0% of plasma cells. Plasma cells are labelled by CD138 (Dako, M115) immunostain and show Lambda (Biogenix, HP6054) restriction. The patient was diagnosed with lambda light chain disease. The M-protein bands for multiple myeloma are usually observed in gamma and beta regions and this patient reflects a rare case of M-spike in the alpha 2 region.

P155

Study of Serum Amyloid A as Lung Cancer Biomarker

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Cancer is the leading cause of cancer death worldwide. Symptoms and radiological features overlap with many other respiratory diseases. Interventional investigations like computed tomography guided fine needle aspiration cytology and bronchoscopy have their own complications. Thus, there is a need for an efficient and non-invasive diagnostic test before the patients are exposed to invasive procedures for tissue diagnosis. Serum biomarkers have been used in diagnosis and prognosis of various diseases. The present study evaluates the use of serum amyloid A (SAA) as a biomarker in the diagnosis of lung cancer. The study included three groups of 20 subjects each: proven lung cancer patients, patients suffering from other diseases and apparently healthy individuals. About 5 ml of blood sample was collected under aseptic conditions and was stored under standard conditions. The serum levels of SAA were measured using ELISA. Statistical analysis was done and cut off value of the serum bio-marker was calculated by receiver operating characteristic curve. Each serum marker was calculated. The median levels of SAA in lung cancer, patients suffering from other diseases and apparently healthy individuals were found to be 9540 ng/ml, 8912.5 ng/ml and 140 ng/ml respectively. With serum levels of 1068 ng/ml as cut off value, SAA had sensitivity of 80% and specificity of 53%. SAA was found to be significantly raised in lung cancer patients as compared to patients suffering from other diseases or healthy controls. SAA could be used as a potential screening tool in the diagnosis of lung cancer.

P156

Altered Expression of Claudin-6 in Breast Cancer and Glioblastoma Multiforme Cells

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Claudin-6 [CLDN6], an integral membrane protein in the tight junction maintains intercellular adhesion and prevents cell invasion. Altered expression of *CLDN6* has been documented in malignancies of carcinoma ovary, gastric adenocarcinoma and hepatocellular carcinoma. Downregulation of Claudins is associated with generation of cancer stem cells from differentiated cells. Limited data is available regarding Claudin-6 expression in breast cancer and glioblastoma multiforme, and that too, without the use of controls. To study the alteration in expression of Claudin-6 gene in breast cancer and glioblastoma multiforme cells with respect to normal breast epithelial cells. Human breast adenocarcinoma cell line, MCF-7, human glioblastoma multiforme cell line, U87MG and human breast epithelial cell line, HBL-100 were used in this study. Expression of *CLDN6* in MCF-7 cells was studied by qPCR ($\Delta\Delta$Ct method). Claudin-6 protein expression in all the cell lines was assessed by indirect immunofluorescence. The median fold change of *CLDN6* in MCF-7 cells was 0.033 ($p < 0.001$). Thus, the expression of *CLDN6* was 30 times lower in MCF-7 cells, compared to HBL-100 cells. Indirect immunofluorescence for Claudin-6 protein showed reduced expression of the protein in MCF7 and U87MG cells compared to HBL-100 cells, in which it was highly expressed. *CLDN6* is down-regulated in MCF-7 breast cancer cell line and U87MG Glioblastoma multiforme cell line. *CLDN6*, thus, may act as a tumor suppressor gene, preventing development of these malignancies.

P157

Comparison Between Tumour Marker Ca15-3 and Uric Acid in Patients with Breast Cancer

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Serum CA 15-3 is regarded as the most suitable breast cancer marker and it became established in the clinical routine worldwide cancer marker. The increased CA 15-3 and uric acid have been associated with the development of breast cancer. The present study was designed to evaluate the correlation between serum uric acid and CA 15-3 tumour marker in patients suffering from breast cancer. The present study was conducted in 253 breast cancer patients and 253 healthy individual. Blood sample from each patient and control groups were taken. Serum sample was used for the estimation of uric acid and serum CA 15-3 tumour marker. Serum uric acid was estimated by the method of Urecase GLDH method and serum CA 15-3 by ELISA method. Serum uric acid and CA 15-3 were significantly higher in breast cancer patients when compared to controls and serum uric acid had a positive correlation with serum CA 15-3 tumour marker. In our study suggests that an increased level of serum uric acid may be due to its protective role in response to increased oxidative stress. CA15-3 was better able to predict breast cancer recurrence than uric acid, but use of both biomarkers together provided a better early indicator of recurrence.

P158

Comparison of Matrix Metalloproteinase-13, a Novel Biomarker with Other Routine Biomarkers in Breast Cancer Patients

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Breast cancer is one of the common and leading causes of cancer death among women worldwide. Matrix metalloproteinase-13 (MMP-13) or Collagenase-3 is emerging as a novel tumor marker for breast carcinoma. It is a member of the matrix metalloproteinase family. MMP-13 protein may promote breast tumor progression. However, its relevance to the progression of human breast cancer is yet to be established. So, the main objective of study was to find out whether MMP-13 can be used as an independent breast cancer biomarker. The study comprised of 50 breast cancer patients as cases and 50 normal age matched healthy controls. MMP-13 levels in serum were estimated by ELISA technique while CA 15-3 and CA -125 levels by ELFA technique. Biochemical parameters – Alkaline Phosphatase, Acid Phosphatase and Calcium levels was determined by using fully automated analyzer using commercially available kits according to manufacturer instructions. MMP-13 level was found highly significant (p value <0.001) in cases than controls. CA15-3 and Acid phosphatase level was also found significant (p value <0.01) while CA-125, Alkaline phosphatase, Calcium levels were non-significant. Therefore, MMP-13 shows high prospect as potential biomarker because of its higher circulating levels in Breast cancer patients.

P159

Oxidative Stress and Antioxidants in Cervix, Breast and Head and Neck Cancer

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In the present decade there is a re-emerging view with competing support from studies at molecular level that raised oxidative stress (OS) is a co-risk factor in many diseases and endogenous antioxidants are relatively more important than exogenous antioxidants. The most convincing and solid support is for cancer. We are herewith presenting evidence for our several to support this concept further. This study included 136 diagnosed cases of cancer (cervix-60, head and neck-30 and breat-76) 60 controls. Endogenous antioxidants (SOD), GPx Cat and GSA were measured in hemolysate and laird peroxide level (ASTBARS), beta-carotene, retinol, alpha lozophsol, and ascorbic acid in plasma by stand as assay procedure. In cervix cancer OS was raised and notably the activity of endogenous antioxidants (SOD, GPx and CAT and GSH) was significantly power. Since these antioxidants act together to regulate cellular redox, we examined if any relationship is followed between OS vs individual antioxidant enzymes and GSH. Most strikingly and interestingly all neck cancer lipid peroxide (LPO) lend was maximum, followed by breast (5.4 ± 31 nmol/ml) and cervix cancer (5.12 ± 0.63). The levels of SOD, GPx and CAT and GSH were also low in head and neck and breast cancer. However no consistent relationship was observed between LPO and endogenous antioxidants. Nutrient antioxidants also did not provide any discernible trend. The raised OS appears to be one of the causative factors in cervix cancer but presently we are unable to conclude the same for head, neck and breast cancer.

P160

Estimation of Serum Fucose in Prognosis of Oral Cancer

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Early diagnosis of cancer helps a great deal in the management of oral cancer patients. Number of protein markers has been employed for this purpose. Recently, conjugated oligosaccharides with proteins and lipids have gained considerable importance in the present genomics and post proteomic period in the diagnosis and prognosis of cancer cases. Tumor cells modulate their surface by increasing fucosylation levels to escape recognition which contribute to several abnormal characteristics of tumor cells. Thus, monitoring serum fucose levels could be a promising approach for the early detection, diagnosis, and prognosis of oral cancer. Serum fucose levels were estimated in 50 control cases and 75 cases of oral cancer by the method of Dische and Shettles as adopted by Winzler at CIMSH, Lucknow. Serum fucose levels were found to be significantly higher in oral cancer cases (46.63 ± 5.29 mg/dl) as compared to the control cases (7.22 ± 0.26 mg/dl). According to standard histopathological grades of oral cancer the mean fucose level increases with severity, from mild [$n = 18$] (15.11 ± 1.07 mg/dl) to moderate [$n = 21$] (31.43 ± 2.83 mg/dl) to severe [$n = 36$] (71.27 ± 3.41 mg/dl) condition. The present study shows that the estimation of serum fucose liberated from fucose conjugated proteins can be a good biomarker which can be used in the diagnosis, staging of oral cancer cases as well as in assessing the prognosis of oral cancer.

P161

Analysis of Circulating Cell Free DNA in Serum as Biomarker in Carcinoma Breast

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In the course of the search for new biomarkers, circulating cell free DNA (ccf DNA) has become a popular target of interest. The ccf DNA can easily be isolated from the circulation and other body fluids of patients, makes it as a non-invasive biomarker of breast cancer. The purpose of this study was to analyze ccf DNA level in breast

cancer patients (pre and post chemotherapy) and also correlate the changes in level with stages of cancer. In this case control study 40 female patients aged 28–80 years who fulfilled the criteria for diagnosis of invasive breast cancer were selected from surgery OPD of S.S. Hospital, BHU Varanasi between March 2014 and July 2015. The estimation of ccf DNA level before and after 2 cycles of neo adjuvant chemotherapy was done by spectroscopic method (Nanodrop). In our study the mean ccf DNA level in case was more than control group ($p < 0.0001$). The mean levels of ccf DNA showed a significant decrease after neo-adjuvant chemotherapy ($p < 0.0001$). In stage II and III both pre chemotherapy patients ccf DNA level was higher than post chemotherapy ($p < 0.0001$). We concluded that ccf DNA is important biomarker in breast carcinoma and can be used for early diagnosis, prognosis and monitoring the chemotherapy response of the patients and also helpful to decrease mortality and morbidity of breast cancer.

P162

Oncofetal Protein Cripto-1 in a new Clinical Role: a Potential Tumor Marker for Oral Cancers

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Cripto1 (CR-1), a member of the EGF-CFC protein family differentially expresses during early embryogenesis. Expression of CR-1 is essential for life in embryogenesis and helps in development of mammary grand and heart in mammals. High expression of immune-reactive protein is also associated with increased number of cancer stem cells. Oral Squamous Cell Carcinoma (OSCC) is one of the commonest cancers in developing countries like India and essentially results in high mortality and morbidity mainly attributed to non-availability of a screening tool or tumor marker for early diagnosis in OSCC patients. We checked tumorigenic property of cripto-1 and its expression in OSCC and evaluated its role as a tumor marker in OSCC. Serum CR1 levels were estimated by sandwich ELISA (R and D Systems™) in 50 biopsy proven OSCC cases and 50 age/sex-matched controls. Serum CR1 levels of the cases before and after standard therapy, according to the stage of the disease, was also estimated. Expression of CR1, at protein level, was estimated by IHC (Immuno-Histo Chemistry) in the cancer tissue. The data were analysed by Mean Whitney and Wilcoxon signed rank test for significance. There is significant ($p = 0.0167$) raise in the serum CR1 level in OSCC patients (mean 459.36 pg/mL) with respect to controls (221.29 pg/mL), which is also significantly reduced ($p = 0.0001$) after completion of therapy in 100% cases. Association between serum Cripto-1 levels and cancer staging and grading was also evaluated and Cripto-1 was found to be associated with early stages of cancer. Pre post Cr-1 levels were decreased significantly in patients with no evidence of disease but not in case of residual disease. There is cytoplasmic positivity in 93% of the cases showed for CR1 in tissue level in IHC. We found that there is a sensitivity of 0.66 and specificity of 0.54 with a cut-off value of 200 pg/ml. We conclude that human serum Cripto 1 is a potential tumor marker for Oral Squamous Cell Carcinoma.

P163

Interleukin-18 as a Potential Biomarker to Determine the Susceptibility and Progression of HBV related Hepatocellular Carcinoma

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Hepatitis B virus (HBV) infection is the main cause of chronic liver disease and accounts for high morbidity worldwide. Immunological factors especially various cytokines have been reported to play an important role in the pathogenesis of HBV infection related hepatocellular carcinoma (HCC). However, studies on the involvement of IL-18 in the pathogenesis and persistence of HBV are limited and thus need to be investigated. The present study has been designed to determine the possible role of IL-18 polymorphism and their serum levels in patients at various stages of HBV infection. A total of 200 patients and 30 healthy controls subjects were enrolled in this study. Polymorphisms at positions −607C/A, and −137G/C in the IL-18 gene were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and real time-PCR-HRM method. Genotype distribution was compared using chi square analysis and the odds ratios (ORs) and 95% CI were calculated to express the relative risk. Serum IL-18 levels were determined with an ELISA kit. In IL-18 (−607C>A), the (CA) heterozygous genotype was found to be a significant protective factor for chronic-active hepatitis ($OR = 0.36$, $p < 0.001$), cirrhosis ($OR = 0.16$, $p < 0.001$) and HCC ($OR = 0.19$, $p < 0.001$) development, among carriers. On the other hand, serum IL-18 levels were significantly increased during the development of HBV related HCC as compared to the control group. These findings suggest that IL-18 levels in different categories of patients show its potential to act as a biomarker of HBV-related disease progression to HCC.

P164

To Assess the Health Status of Diabetic Patients through Anthropometric Measurement

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Diabetes mellitus is a disease characterized by high blood glucose concentration in the blood and alteration in carbohydrate, protein and fat metabolism. People have greater risk of diabetes due to improper dietary practices, unhealthy life style, and lack of physical exercise. We assess the nutritional status among diabetic subjects and draw a guideline for a healthy life style which is valuable in treatment or prevention of diabetes. Multistage stratified random sampling technique was used for selecting 100 samples in both 48

males and 52 females and an interview schedule was developed to collect information regarding socioeconomic profile, dietary pattern, nutritional status etc. The anthropometric measurements of all the subjects were determined. Dietary intake between males and females diabetic were highly significant but age, BMI, meals per day etc. between males and females diabetic were insignificant. Consumption of high fat and carbohydrate diet was seen as the major contributing cause of disease in both males and females.

P165

Estimation of Iron Status in Paediatric Patients with Beta Thalassemia and Sickle Cell Disease

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Blood transfusion is the mainstay of supportive treatment in patients with thalassemia and sickle cell disease which is life-saving at the same time can cause overt side effects. We investigate iron status and find correlation with number of blood transfusions in patients with thalassemia and sickle cell disease. This comparative study is carried out in the Paediatrics department and lab investigations being carried out in the clinical biochemistry laboratory and RIA center for one year. 30 cases of thalassemia and 30 cases of sickle cell disease were enrolled for study. Serum iron and TIBC estimated in semi-autoanalyzer by colorimetric method and ferritin estimated by radioimmunoassay method. In patients with thalassemia mean ± S.D. of iron, TIBC and ferritin found to be 184.73 ± 26.96, 235 ± 33.39, 1103.16 ± 450.26 and in sickle cell disease this results are 148.36 ± 24.16, 221.16 ± 56.29, 673.34 ± 356.26 respectively. There is statistically significant positive correlation of number of transfusion with ferritin in both group of patients ($p < 0.05$). Estimation of iron status is important in transfusion dependent patients as management for iron overload and its untoward effect can be done at the earliest with proper monitoring.

P166

Putative Metabolic Effect of Plant Oxysterol in Diabetic Rat

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Plants have been known to synthesize brassinosteroid family of hormones, although in low abundance. Recent studies using homocastasterone (ketoisoform) and an earlier study using 28-homobrasslinolide (aldoisoform) assessed their influence on glucose homeostasis and in testicular steroidogenesis in normal and diabetic male albino wistar rats employing oral feed regimen and determining blood and tissue levels of specific biomarkers. Attenuation of blood glucose level and elevation in testicular testosterone level were noted through both investigations. Reduced plasma/ tissue transaminase activity and augmented hepatic glycogen content were caused by the ketoisoform. Renormalization of tissue architecture was noted in selective histological sections following administration of these compounds to experimental rat, in both studies. Decrease in blood/ tissue urea content and increase in plasma/ tissue total protein content were also noted. The comparative study suggests that phytosteroid induced hepatic glycogen and systemic protein synthesis, reduced circulating glucose level and yielded glucose homeostasis. Prosteroidogenic potential associated with these compounds is also established. Subliminal intake of phytosteroids therefore can impact endogenous steroid synthesis and energy homeostasis.

P167

Protection of Liver Injury by Vitamin C, E and GSH after Methomyl Toxicity in Rat

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Methomyl (Lannate), a carbamate pesticide induces liver injury by increasing lipid peroxide, suproxide dismutase, and inhibiting microsomal cytochrome P_{450} which is prevented by supplementation of vitamins. We study effect of supplementation of vit.C, E, and GSH on lipid peroxide and superoxide dismutase, mixed function oxidase in methomyl treated rats. Adult male rats (weighing 200–230 g) were divided into 4 groups each of 6 animals. Animals from group 2, 3 and 4 were given a dose of 1, 2 and 4 mg methomyl/ kg body weight (BW) i. p., respectively for 3 consecutive days. Second set of experiment adult male rats were divided into 4 groups. Animals from group 2, 3, 4 were injected selected dose methomyl (4 mg/kg BW) for 1, 3 and 5 successive days. Third set of experiment adult male rats were divided into 5 groups. Animals from group 2 were injected methomyl 4 mg/kg BW. Animals from group 3, 4, and 5 were injected methomyl (4 mg/kg BW) along with Vitamin C, E and GSH (100 mg/kg bw each) respectively. Group-1 of each category received an equivalent amount of saline as control. We used one-way analysis of variance and Tuckey Krammerpost test. Increased dose and duration of methomyl treatment to rats increases the microsomal LP and SOD. Supplementation of vitamin C, E and GSH (100 mg/kg bw each) to methomyl pretreated groups received in saline water separately and observed that microsomal LP and SOD was significantly increased in methomyl ($P < 0.01$and $P < 0.05$) treated rats and decreases in supplementation of vitamin C, E and GSH to methomyl- pretreated rats. In methomyl + vitamin E ($P < 0.05$and ns), in methomyl + vitamin C ($P < 0.05$and ns), in methomyl + GSH ($P < 0.05$and ns) treated rats, respectively as compared to control rats. Group-1 injected 0.9% saline and served as control in all the experiment. Alteration in microsomal mixed function oxidases observed in Methomyl toxicity. Protection of liver is observed after supplementation of vitamin C, E and GSH on mixed function oxidases.

P168

Lipid Profiles of Postpartum Rats administered Dry Lake Salt (*Kanwa*)

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Peri-partum cardiomyopathy (PPMC) is a rare but devastating cardiac failure of indeterminate etiology occurring in late pregnancy or early puerperium. Dry lake salt (Kanwa) is usually consumed in many parts of Nigeria as laxatives. In the Northern Nigeria, with a high prevalence of PPMC, it is consumed postpartum in large quantities as a traditional practice. This work investigated the effect of kanwa on serum lipid profile, blood pressure and body weight of postpartum rat administered graded doses of kanwa. The female rats were grouped into 4 of five animals each postpartum. The rats in the groups were administered 0 mg/kg (control), 100 mg/kg, 200 mg/kg, and 300 mg/kg body weight respectively of Kanwa orally for four weeks. The results indicated a significant decrease in HDL-C when compared with the control ($P < 0.01$) while all other lipid profile parameters (TAG, T.CHOL, VLDL-C, and LDL-C) tested were found to have no significant ($P > 0.05$) different from the control. However, there is a no significant ($P > 0.05$) increase in atherogenic index of the treated group as compared with the control. Moreover, blood pressure as well as body weight results are all considered not significantly different from control ($P > 0.05$). The result indicated that kanwa may play a significant role in the pathogenesis of the PPMC.

P169

Maternal Serum and Cord Blood Vitamin D levels in Term Normotensive and Pre-Eclamptic Patients

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There is a growing concern that neonates are entering the world with a Vitamin D deficit which has significant effects on innate immune function and childhood bone development that begins in-utero. Recent studies in Western countries have demonstrated a high prevalence of Vitamin D deficiency in mother-neonate pairs at birth. To evaluate and correlate maternal serum and cord blood Vitamin D levels in term normotensive and pre-eclamptic pregnant women. Case-control study involving 100 pregnant women (50 pre-eclamptic women and 50 normotensive), who were evaluated for maternal and cord blood Vitamin D levels. Additional factors like maternal and cord blood calcium, phosphorus, and ALP and PTH levels were also assessed. Pre-eclamptic group had lower median Vitamin D levels (3.9 ng/ml) as compared to normotensive group (9 ng/ml). Neonates born to pre-eclamptic mothers had lower median cord blood Vitamin D levels (4.4 ng/ml) as compared to their normotensive counterparts (7.25 ng/ml). Mean maternal calcium levels were relatively lower in pre-eclamptic women (8.50 ± 0.80 mg/dl) as compared to normotensive women (8.89 ± 0.55 mg/dl) ($p = 0.006$). Similarly, the cord blood calcium levels were significantly lower in neonates of pre-eclamptic women (8.92 ± 1.03 mg/dl) vs. neonates of normotensive women (9.64 ± 0.84 mg/dl) ($p < 0.001$). The difference in PTH and phosphorus levels was insignificant. The findings of the study suggest that pre-eclampsia is indeed associated with lower Vitamin D levels. Maternal Vitamin D concentration plays a crucial role in maternal and neonatal calcium homeostasis; therefore, the infants of mothers with Vitamin D deficiency during pregnancy have low serum calcium concentrations at birth.

P170

Grape (*Vitisvinifera*) Extracts Protect against Radiation-Induced Oxidative Stress and DNA Damage

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Ionizing radiation (IR) causes oxidative stress through the overwhelming generation of reactive oxygen species (ROS) in the living cells leading further to the oxidative damage to biomolecules. Grapes (*Vitisvinifera*) contain several bioactive phytochemicals and are the richest source of antioxidant. In this study, we investigated and compared in vitro antioxidant activity and DNA damage protective property of the grape extracts of four different cultivars, including the Thompson seedless, Flame seedless, Kishmishchorni and Red globe. The activities of ascorbic acid oxidase and catalase significantly ($p < 0.01$) differed among extracts within the same cultivar, while that of peroxidase and polyphenol oxidase did not differ significantly among extracts of any cultivar. In vitro antioxidant activities were assessed by ferric-reducing antioxidant power (FRAP) assay and ABTS. The superoxide radical-scavenging activity was higher in the seed as compared to the skin or pulp of the same cultivar. DNA damage was evaluated in acellular system using pBR322 plasmid relaxation, as well in genomic DNA from blood. Grape extract was able to effectively scavenge free radicals in vitro. It could significantly prevent radiation-induced DNA damage. Furthermore, the protective action of grape depends on the source of extract and type of the cultivars.

P171

Status of Vitamin D in General Population of Tamil Nadu

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Vitamin D is considered to be very important for maintaining a proper skeletal system in human beings. One of the major physiological functions of vitamin D is to maintain the serum concentrations of calcium and phosphorus. Data on the vitamin D status

of the population in India is minimal and being a tropical country, the vitamin D deficiency is presumed to be rare and uncommon. This study was a retrospective study carried out to assess the levels of vitamin D, serum calcium, phosphorus and alkaline phosphatase levels in a south Indian state population. A total of 1394 healthy subjects (822 females and 572 males) were studied for their serum calcium, phosphorus, alkaline phosphatase and vitamin D levels. Vitamin D levels were deficient in all the study subjects. There was no significant difference in the vitamin D levels between males and females. The subjects were divided into four age (years) groups, Group I (1–18), Group II (19–40), Group III (41–60) and Group IV (> 60). The vitamin D levels in group I females was found to be the lowest and the levels were significantly low when compared to group I males. The serum calcium level showed a decreasing trend with age in both males and females. The study reveals the serious nature of the vitamin D deficiency status in our general population. Steps need to be taken to create awareness among people and to suggest dietary and life style changes to overcome the deficiency condition. Health care professionals need to be sensitized to this issue and trained to identify the early symptoms of the vitamin D deficiency so that supplementation may be provided to such people avoiding long term and permanent complications.

P172

Study of Association of High Fibre Diet to Blood Pressure and Cardiac Risk Ratio: A Case Control Study

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In the present day changing lifestyle, it is necessary to understand that a simple habit of consumption of salad can be effective in controlling blood pressure and reducing cardiac risk. The phytochemicals present in vegetables and salads, reduces inflammation occurring in the arterial wall and prevent blood cells from sticking together. Cardiac risk ratio (CRR) has a better predictive power of cardiovascular disorders than the individual parameter. Hence this study was designed to know the association of high fibre diet to blood pressure and CRR. To understand the association of high fibre diet to blood pressure and cardiac risk ratio 150 participants were selected based upon the exclusion criteria. The dietary habits and demographic information was collected in a pre-validated questionnaire. The participants were divided in to four groups, namely high fibre consuming men and women and low fibre consuming men and women. Their blood pressure was measured and serum samples were analyzed for lipid profile. The results were analyzed statistically using graph pad prism. Amongst the 255 persons interviewed, only 28 % were found to be regularly consuming high fibre diet. There is a non-significant higher prevalence of cases with CRR > 5 in men and women consuming low fibre than their counterparts. The 95% Confidence Interval for the odds ratio for high fibre consumption and hypertension in women is significant. The high fibre diet has a significant association to blood pressure in women. It decreases CRR in men and women. It is necessary to increase awareness about benefits of high fibre diet.

P173

Change in Status of Sunshine Vitamin in Children: Still Lots to be Done

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Half of the skeletal mass at maturity is accumulated during childhood and adolescence, making optimal bone mineral health in children very important. Vitamin D earlier known for its role in calcium homeostasis and bone metabolism is now known to influence expression of >200 genes, since its receptors have been found in many tissues. Vitamin D requirement can be much more than synthesized in the body or taken in the diet of north Indians resulting in its deficiency. Moreover therapeutic use of vitamin D is becoming common. With the aim of studying deficiency in children and change in status over past years, the present study was conducted. Thirty three hundred children for analysis of Vitamin D deficiency due to back pains, unexplained muscle pains, general fatigue were advised 25 (OH) D investigations in years 2010–2013. Data was collected from Biochemistry lab. 25(OH) D analyzed on Cobas 6000. Patients were divided into four groups I-IV (0–1 yrs, 2–6 yrs, 7–12 yrs, and 13–18 yrs respectively). 25(OH)D levels (ng/ml) classified as: severe deficiency: <4, deficient: <20, insufficient: 20–30, sufficient: >30, excess: >70. Number of children reporting for investigation increased every year. Vitamin D deficient children decreased significantly from 73.8% to 56.8% over 4 years, 15% subjects had severe deficiency, which decreased to 8–6% by 2013. Higher number of older children (80–85%, group III, IV) were found deficient compared to younger ones. 11.3% children had insufficient levels which increased to 18% in 4 years. Number of children having sufficient levels also increased from 11–3% to 18% in 2013, 3–8% had excess levels now. Vitamin D deficiency in symptomatic children decreased over four years. Decreasing prevalence of deficiency in children shows impact of supplementation. Same impact resulted in large no of children falling in excess levels group. Regular monitoring and correct therapeutic dose adjustment especially in children is important.

P174

Estimation of Liver enzymes and Zinc in Protein Energy Malnourished Children at Nutritional Rehabilitation Centre of Bhopal

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Malnutrition is the cellular imbalance between supply of nutrients and energy and the body demand for them to ensure growth, maintenance and specific functions. The present study was undertaken to evaluate the role of Liver enzymes and zinc in the pathophysiology of PEM children. 60 malnourished children in the

age group of 6 months to 5 years were selected and compared to 60 healthy age and sex matched controls. The liver enzymes alanine transaminase [ALT], aspartate transaminase [AST] and alkaline phosphatase [ALP] were measured by IFCC method [kinetic] and zinc was measured by colorimetric method. There was significant increase in the alanine aminotransferase and aspartate aminotransferase in cases as compared to controls. On the other hand, there was significant decrease in the serum alkaline phosphatase and zinc levels. Decreased dietary intake of antioxidant mineral zinc leads to increased oxidative stress. This causes lipid peroxidation of membrane lipids. Peroxidative attack on plasma membrane of hepatocytes causes extensive damage so that enzymes AST and ALT are able to leak out causing rise in plasma levels. Alkaline phosphatase is an important zinc containing metalloenzyme. Its decreased activity may due to low dietary intake of zinc as well as proteins. Thus malnourished children have poor growth, skeletal maturation and immunocompetence.

P175

Study of Anemia in CKD Patients with Special Reference to Hepcidin

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Anemia is a major complication of CKD. The major cause of anemia in CKD is erythropoietin deficiency. But hyporesponsiveness and resistance to ESAs emerging. It has been hypothesized that inflammation may play an important role in anemia of CKD. Although serum ferritin and transferrin saturation (TSAT) are commonly used as biomarkers for iron status in CKD patients, these markers are not sensitive enough to distinguish functional iron deficiency from iron overload. Recently, Hepcidin, an acute phase reactant protein produced in the liver, is thought to be central regulator of body iron metabolism. We studied anemia in 100 adult non dialysis dependent CKD (Stage 3–5) patients in a hospital based cross sectional study. Hepcidin levels, ferritin levels and hsCRP were elevated in patients of CKD with anemia. Hepcidin levels increased as CKD progressed through stage 3–5 (p trend = 0.015) but did not correlate with estimated glomerular filtration rate. Hepcidin correlated positively with ferritin ($p < 0.0001$) and % transferrin saturation ($p = 0.0217$) and negatively with erythropoietin levels ($p = 0.0258$) but did not correlate with either hsCRP or eGFR. Haemoglobin correlated significantly and positively with eGFR ($p < 0.0001$). Haemoglobin correlated negatively with ferritin and hepcidin in univariate model, but did not correlate with either of them in multivariate model. Iron status influenced hepcidin levels of patients. We divided patients into different groups according to iron status based on study done by Mercadel et al. we observed that while absolute iron deficiency (TSAT < 20%, Ferritin < 40) is associated with down-regulation of hepcidin. Iron status of patients also influences interaction between hepcidin and haemoglobin. Hepcidin correlated negatively in patients with sufficient iron status but nearly correlated positively with haemoglobin in patients with absolute iron deficiency.

P176

Temporal Expression of Genes Involved in Folate Metabolism and Transport During Placental Development

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Folate is an essential micronutrient during pregnancy and placental development. However, the expression of genes related to folate transport and metabolism during the advancing gestation and pregnancy complications is not well known. Considering this, we evaluated the expression of folate transporters and enzymes involved in folate metabolism in placenta in different trimesters of normal pregnancy and pregnancy related disorders viz., preeclampsia and NTD. The expression of folate transporters and enzymes involved in folate metabolism in placenta of different trimesters of normal pregnancy and pregnancy related disorders were studied by 2-step RT-PCR. Folate levels were estimated by microbiological assay using *L. casei*. Significant changes in levels of placental folate-metabolising enzymes were found in both physiological and pathological groups of pregnant women. In NTDs, there is an adaptive up-regulation of folate transporters mainly *RFC* and *FRα*. *MTHFR* expression has strong positive correlation with folate levels in placenta. Overall, folate plays an important role in development of placenta during normal healthy pregnancy. Deficiency of folate or alteration in expression of enzymes involved in folate metabolism might result in changes in folate levels in placenta and might be associated with pregnancy complications such as preeclampsia and NTDs.

P177

Effect of Iron Supplementation in Pregnancy on Iron Status, Oxidative Stress, Inflammation and Insulin Resistance

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The prevalence of anemia in pregnancy is about 65–75% in India, which is associated with complications like prematurity, low birth weight, maternal mortality and morbidity. Hence universal recommendation is to provide 60 mg elemental iron daily. But studies have shown that providing excess iron can cause iron overload which may lead to oxidative stress and further metabolic derangements. So providing iron may be beneficial in anemic women but it may

increase risk in non-anemic women. To assess the effect of iron supplementation on hematological parameters, oxidative stress, low grade inflammation and insulin resistance in anemic and non-anemic pregnant women. Forty non-anemic and forty anemic pregnant women were recruited at 12 weeks of gestation. They were given iron supplementation depending on their hemoglobin levels. Blood samples were collected during recruitment and at 28 weeks of gestation for the analysis of parameters. After iron supplementation in non-anemic women, there was no significant difference in hematological parameters. MDA/TAS ratio, HOMA-IR and hsCRP were significantly increased. Ferritin and fasting glucose did not show significant change. After iron supplementation in anemic group, significant increase was observed in Hb, hematocrit, RBC count and RBC indices. Ferritin, and HOMA-IR were significantly increased and hsCRP decreased significantly. Iron supplementation improved hematological status and decreased inflammation in anemic pregnant women but it increased oxidative stress, insulin resistance and inflammation in non-anemic pregnant women besides not improving their hematological status. Hence iron supplementation should be considered with caution in non-anemic pregnant women.

P178

Amla Prevents the Redox Imbalance and Inflammatory Response in High Fat Fed Hypothyroid Rats

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The aim of this study was to evaluate the Preventive effect of Amla (*Emblica officinalis*) on Redox Imbalance and inflammatory response in High fat fed hypothyroid rats. Five month old female wistar rats ($n = 80$) were used and divided into 2 major study groups. The study group consists of untreated groups (Study-1) and Treatment groups (Study-2). Study-1 were further divided into 4 sub-groups as control (Normal chow diet), Hypo (treated with PTU), HFD (High fat diet) and Hypo + HFD. The Study 2 was divided as in study-1 and treated with Amla extract (AE) 100 mg/kgbw/day, along with the routine treatment in all the sub groups. The experiment was carried out for 12 weeks and divided into two phases: Phase1 for (4 weeks) and Phase 2 for following (8 weeks). In study-1, Hypo and HFD groups show decreased GSH, TAC levels and elevated GPx, MDA, TNF-α and CRP levels. In study-2, elevated GSH, TAC levels and decreased GPx, MDA, TNF-α and CRP levels were observed in blood Besides, the protein expression by western blot showed decreased TNF-α and p38 expression in the Study-2 (treatment groups) when compared with Study-1 (Untreated groups). The treatment with AE showed decrease in mRNA expression (RT-PCR) of cyclooxygenase-2 (COX-2) and NADPH oxidase-4 (NOX-4). High fat fed hypothyroid rats show elevated oxidative stress and inflammatory response in both biochemical and molecular levels. Treatment with Amla Extract (AE) prevented hypothyroid induced oxidative stress and inflammation. The Amla extract showed a preventive effect in maintenance of Redox balance and inflammatory response in hypothyroid induced oxidative stress and inflammation.

P179

Association of Cord blood Hemoglobin and Ferritin Concentration with Maternal Anemia

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In a developing country like India, Anemia in pregnancy impose a major challenge in obstetrics with moderate prevalence. Although multifactorial causes implicated, nutritional deficiencies (micronutrients-especially iron deficiency) is by far most common cause of maternal anemia in India. One good index of iron store is measurement of serum ferritin concentration in the absence of acute and chronic inflammatory conditions. The aim of the present study was to correlate the pre-delivery maternal hemoglobin concentration with cord blood hemoglobin and ferritin concentration of their newborns. This case control study involved 108 pregnant women and their newborns. Forty-two pregnant women were anemic and 66 non-anemic. Hemoglobin concentration was assessed in the pregnant women. Cord blood was collected EDTA and plain tubes for full blood count analysis and ferritin assay. Hemoglobin concentration was assessed by automated equipment and serum ferritin assay was done by automated CLIA method. With the set cut off for hemoglobin concentration for categorizing anemic and non-anemic pregnant women, the cord blood hemoglobin concentration for anemic groups was found to be significantly lower than in the non-anemic group ($p < 0.001$). The cord blood serum ferritin concentration was also significantly low in newborns of anemic mothers as compared to newborns of non-anemic mothers ($p < 0.01$). Significant association was found between maternal anemia and cord blood serum ferritin concentration ($p < 0.05$). In conclusion, it was found in this study that maternal anemia has significant effect on cord blood hemoglobin and ferritin concentrations.

P180

Correlation of Thyroid Status, Iron, Folic Acid and Vitamin B12 Levels in Pregnancy

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Iron deficiency is quite prevalent in pregnancy. Role of iron deficiency with thyroid status in pregnancy has not been studied in detail. We assessed the levels of iron, folic acid, vitamin B12, and thyroid hormones and studied the association of thyroid profile with iron levels in first trimester of pregnancy. Forty pregnant women who didn't start any supplementation were recruited from the obstetrics and gynecology out-patient department. Forty age matched controls

were recruited from the residents and staff of the hospital. Thyroid profile, iron, folic acid and vitamin B12 levels were measured in both the groups. The association was seen between iron levels and thyroid stimulating hormone (TSH) levels. Levels of iron, folic acid, vitamin B12 are significantly low in first trimester pregnant women. Further, the increased TSH levels are negatively correlated with low iron levels. Screening of iron, folic acid and vitamin B12 levels in first trimester itself will be beneficial to prevent the complications of pregnancy. Further, hypothyroidism also present and associated with iron deficiency. So, early diagnosis of these deficiencies will be useful to start giving supplements to avoid unwanted effects in pregnancy.

P181

Association of Vitamin B_{12} and TSH Concentrations with Lipid Levels in the Subjects Under Preventive Health Check Up: A Pilot Study

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High prevalence of hypothyroidism and Vit.B_{12} deficiencies was observed in subjects attending preventive health check-up. A study was designed to find an association of hypothyroidism and vit.B_{12} deficiency with serum lipids. Three month's data of 1805 subjects between 20 to 80 yrs of age from Pune was analyzed (66.6% males and 33.4% females). Fasting serum was measured for Lipids, TSH and Vit. B12 using Architect ci4100. Two third of the subjects (66.1% males and 53.3% females) were vit.B12 deficient. Deficiency was less severe in older subjects. 12.2% of the subjects had elevated TSH above 4.0 mIU/Land the frequency was almost double in females (17%) than males (9.0%). There was significant positive association of Vit. B_{12} with HDL-c, inverse association with Triglycerides and LDL-c and no association with cholesterol at all age levels. Dyslipidemia was maximum in males (53.2%) between 36 to 50 yrs and was age dependent in females being maximum (15.5%) above 50 yrs. There was no association between vit.B12 and TSH with each other. Prevalence of Vit. B_{12} deficiency was more than hypothyroidism and was higher in males than females, while, hypothyroidism was more in females. Dyslipidemia was highest in males between 36 and 50 yrs whereas, in females above the age of 50 yrs. Vit.B12 had significant positive association with HDL-c and inverse association with LDL-c and Triglyceride and no association with cholesterol. There was no association between vit. B_{12} and TSH.

P182

Holotranscobalamin (Holotc) an Optimal and Early Marker of Vitamin B12 Deficiency and Changes in Cobalamin Homeostasis - A Silent Epidemic with Serious Consequences

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The larger part of vitamin B12 (70 - 90%) is inactive and cannot be used in the metabolism. The active part, which can be used by tissues directly, is carried in a complex called HoloTC and is available for tissue uptake. To assess the usefulness of serum HoloTC compared with total vitamin B12in patients with clinical suspicion of vitamin B12 depletion. Serum samples were randomly selected from 85 patients (52 males, 33 females; age range 12–69 years) referred to the Clinical Biochemistry Laboratory, Sir Ganga Ram Hospital for the assessment of vitamin B12 status. For each patient, both serum total vitamin B12 and holoTC level was determined by micro-particle enzyme immunoassay on Architect1000i SR analyzer (from Abbott, USA). We divided the patients in 4 groups on basis of their Total B12: <250, 251–350, 351–450 and >450 pmol/l respectively. The groups contained 25, 25, 20 and 15 patients respectively. In these groups the number with decreased HoloTC (< 35 pmol/L) were respectively 21 (84%), 17 (68%), 6 (30%) and 3 (20%). So a considerable number of patients with normal Total B_{12} have low HoloTC. It can be recommended that holoTC either alone or in combination with total vitamin B12, can be used for the screening/diagnosing vitamin B12 deficiency for the majority of the population. Holo TC may prove most useful if the aim is to monitor a population with a borderline suboptimal vitamin B 12 levels. Prevalence of Vitamin B12 deficiency in a population can be estimated by measuring HoloTC. When a deficiency of vitamin B12 is diagnosed at an early stage, many cases can be treated or prevented, with beneficial effects on individual outcomes and subsequent potential reductions in health-care costs.

P183

Effect of Paternal Folate Levels on Expression of Various Imprinting Genes and Folate Transporters in Placenta and Fetal Organs

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Epidemiological studies suggest that paternal diet can influence offspring health. Since, both paternal and maternal alleles provide their DNA sequences equally to a fertilized egg, it is possible that the DNA methylation pattern of fetus may be influenced by the paternal allele through alterations in sperm epigenome. Therefore, we hypothesize that paternal dietary supply of methyl donors will alter epigenetic reprogramming in sperm which might affect expression of imprinting genes in placenta and fetal organs. We examined the effect of paternal folate deficiency on some of the imprinting genes in C57BL/6 mice. Based on the diet, male and female mice were divided into four groups PDMD, PDMN, PNMN and POMN (paternal folate-deficient, PD, paternal folate normal, PN, paternal folate over-supplementation, PO, maternal folate-deficient, MD, maternal folate normal, MN) for six to eight weeks. They were mated and grouped accordingly: PDxMD, PDxMN, PNxMN, and POxMN. Vaginal plugs were detected and females were sacrificed on day 21 post-conception. The placental and fetal tissues were collected. There was a significant decrease in expression of *IGF2* in brain and liver of fetuses of PDMD group as compared to PNMN group. *H19* expression was found to increase in fetal brain in PDMD group which is a negative regulator of growth. FRα expression was significantly higher in placenta of PDMD group as compared to normal whereas RFC expression was found to be increased in tissues of fetus of PD group. Various physical malformations such as fused placentas and small fetuses were also seen in pregnancies sired by PD groups. These results show

that paternal folate deficiency at mating can influence the imprinting genes and folate transporters expression in placenta and fetal organs.

P184

Increased Synthesis of Folate Transporters Regulates Folate Transport in Conditions of Ethanol Exposure and Folate Deficiency

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Excessive alcohol consumption and dietary folate inadequacy are the main contributors leading to folate deficiency. The present study was planned to study regulation of folate transport in conditions of folate deficiency (FD) and ethanol exposure in human embryonic kidney cell line. Also, the reversible nature of effects mediated by ethanol exposure and folate deficiency was determined by folate repletion and ethanol removal. For ethanol treatment, HEK293 cells were grown in medium containing 100 mM ethanol and after treatment one group of cells was shifted on medium that was free from ethanol. For FD treatment, cells were grown in folate deficient medium followed by shifting of one group of cells on folate containing medium. FD as well as ethanol exposure resulted in an increase in folate uptake which was due to an increase in expression of folate transporters i.e. reduced folate carrier (RFC), proton coupled folate transporter (PCFT) and folate receptor (FR), both at the mRNA and protein level. The effects mediated by ethanol exposure and folate deficiency were reversible on removal of treatment. Promoter region methylation of folate transporters remained unaffected after folate deficiency and ethanol exposure. As far as transcription rate of folate transporters is concerned, an increase in rate of synthesis was observed in both ethanol exposure and FD conditions. Additionally, mRNA half-life of folate transporters was observed to be reduced by FD. An increased expression of folate transporters under ethanol exposure and FD conditions can be attributed to enhanced rate of synthesis of folate transporters.

P185

A Randomised Controlled Trial to Assess The Effect of Vitamin D Supplementation in Steroid Sensitive Nephrotic Syndrome

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Nephrotic syndrome (NS) is associated with immune dysregulation and osteoporosis. Vitamin D has modulating effects on the immune and skeletal systems. Our aim is to assess the effect of vitamin D supplementation on bone biochemistry, bone mineral density (BMD) and on relapse rate and steroid requirement in patients with steroid sensitive nephrotic syndrome (NS), over a period of 6 months. Open labeled randomized controlled trial, with 30 patients (age: 2–10 years) in each arm, to yield >90% power. The test group receives vitamin D and calcium supplementation. Standard treatment of NS is continued. Serum creatinine, albumin, calcium, phosphate, alkaline phosphatase, and 25-hydroxy-cholecalciferol (25(OH)D); urine protein/creatinine and calcium/creatinine ratios are documented at study entry, 1 month and 6 months. Renal ultrasound and lumbar BMD are performed at study entry and 6 months. The number of relapses and cumulative steroid dosage during study period is documented. Till date, 40 patients have undergone randomization while 26 have completed the study (test group: 12, controls: 14). Interim analysis indicates that between groups, at study entry, there was no difference in NS type, mean age, 25(OH)D levels or lumbar BMD. The mean initial 25(OH)D level was 6.4 ng/ml which correlated significantly with serum albumin ($r = 0.39$, $p = 0.013$), but not with NS type or BMD. The mean difference over 6 months in 25(OH)D levels was +20.1 ng/dl in the test group and +0.9 ng/ml in the control group ($p = 0.002$), while mean difference in BMD was +8.7% and +3.3% ($p = 0.27$) respectively. Four (33%) patients in the test group had relapses compared to 10 (71%) in the control group ($p = 0.1$). On study completion we expect to understand whether optimization of serum vitamin D levels reduces relapse rate and steroid requirement or maintains BMD in children with NS, in the short term. This will help in formulating definitive guidelines for the requirement of such supplements, in this chronically relapsing disease.

P186

Association of Chronic Liver Disease and Vitamin D

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Chronic Liver Disease (CLD) is defined as long term process of progressive destruction and regeneration of liver and with advancement ultimately leads to hepatic fibrosis, scarring, and cirrhosis. 25(OH) Vit-D is synthesized in liver and in CLD Vit- D homeostasis is impaired. Available reports indicate an altered level of 25(OH)Vit-D in these subjects. CLD extends to hepatic cirrhosis and fibrotic changes, liver function is also altered in these subjects. Active metabolite of Vit-D 1, 25(OH) D3 is involved in calcium and phosphate metabolism and exerts a large number of biological effects. Nearly 43.6% Vit-D deficient subjects developed CLD and neurological disturbances. Deficiency of Vit-D has been found to be associated with cholestatic liver disease like primary biliary cirrhosis and some patients also developed osteoporosis due to altered calcium and Vit-D metabolism. In this study, we have estimated serum 25(OH) along with a routine liver function test and plasma fasting glucose in 45 patients of CLD of various types, 35 controls were selected from population outside the hospital. A large number of control subjects were deficient of 25(OH) Vit-D with normal LFT and fasting glucose. The test cases were compared and a low level of 25(OH) D was observed in subjects of CLD. Results will be discussed.

P187

Assessment of Hb, Iron and TIBC in Chronic Kidney Disease

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Chronic kidney disease is when one suffers from gradual and usually permanent loss of kidney function over time. Anaemia is a major factor that limits the quality of life in chronic kidney failure patients and may affect their morbidity and mortality. Insufficient production of erythropoietin from the failed kidneys is the major cause of anaemia in this population. The objective of the study is to assess haemoglobin (Hb), serum Iron, Total Iron Binding Capacity (TIBC) levels in assessing and monitoring body iron stores which can influence iron management and treatment in patients with CKD. 50 subjects were taken and divided into group 1: Healthy controls ($n = 25$); group 2: CKD patients ($n = 25$), taken from Indira Gandhi Medical College and Hospital, Shimla. Mean age of CKD patients 47 ± 13. Levels of Hb, Iron, TIBC were 7.4 ± 2.1, 54 ± 9.6, 236 ± 61 respectively in CKD patients. Mean age of healthy controls was 50 ± 20. Mean Hb, Iron and TIBC 14.3 ± 2.2, 115 ± 55, 346 ± 75 respectively, in healthy controls. Further Mean Hb, Iron, TIBC were 6.7 ± 1.8, 58.8 ± 11.1, 221 ± 19.3 in male patients of CKD whereas mean Hb, Iron, TIBC were 6.6 ± 2.9, 42.2 ± 13.7, 236 ± 51 in females CKD patients. Hb and serum iron levels decrease both in male and female patients. Lower value is obtained in females. TIBC value is within normal range or slightly increases in both. Pre and post therapeutic intervention can be decided on the basis of Hb, iron and TIBC level.

P188

Correlation of Gallstone Formation and Serum Iron Levels

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Gallstones are one of the most common problem associated with the gall bladder, affecting millions of people throughout the world. Bile is excreted from liver and gall bladder in stomach for digestion. After digestion, if the gall bladder is not emptied out completely, the bile that remains in the gall bladder can become too concentrated with cholesterol and gallstones are created. Cholesterol and calcium bilirubinate are the two main substances involved in gallstone formation. Gallstones derived from bile consist of mixture of cholesterol, bilirubin, with or without calcium. Based on their chemical composition gallstones found in the gall bladder are classified as cholesterol, pigmented or mixed stones. Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased gall bladder bile cholesterol saturation and promotion of cholesterol crystal formation. We attempt to establish a correlation with gall stones and decrease serum iron levels. This study was a prospective cohort study which included 100 consecutive patients admitted in the ward of K.J. Somaiya Hospital and Research Centre, Mumbai with imaging studies suggestive of Cholelithiasis. The Gall stone surgically removed was crushed with mortar and pestle and then analyzed for cholesterol, calcium, phosphate and bilirubin (pigment). Serum sample was analyzed for Cholesterol, iron and iron binding capacity. 86% patients had increased cholesterol levels and 93% had decreased serum Iron levels. The most common type of gall stone was found to be cholesterol type (76%) of gall stone followed by mixed (19%) and pigment (5%) gall stones. Serum cholesterol levels were found to be raised in majority of the patients (86%) which might be a predisposing factor for cardiovascular diseases, etc. Similarly serum iron was found to be low in majority of the patients (93%) indicating iron deficiency as a cause of gall stone formation

P189

Correlation of Elevated Serum Ferritin, Uric Acid and, Malondialdehyde in Non-Alcoholic Fatty Liver Disease

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Non- alcoholic fatty liver disease is an increasingly recognized condition that may progress to end-stage liver disease. The clinical implication of nonalcoholic fatty liver disease are derived mostly from its common occurrence in the general population and it's potential to progress to liver cirrhosis and liver failure. We aim to determine the levels of serum ferritin, uric acid and, malondialdehyde in non-alcoholic fatty liver disease patients and their comparison with healthy controls. The present study included 150 non-alcoholic fatty liver disease patients (75, diabetes mellitus and 75 obesity) and, 75 healthy controls, age between 25 and 75 years, both male and female. Following investigations were carried out: 1) Estimation of serum ferritin by C.L.I.A (Elisa) method, 2) Estimation of serum uric acid by uricase peroxidase method, and 3) Estimation of malondialdehyde by Jean CD *et.al*, method. This study showed significantly increased serum levels of ferritin- $p < 0.001$), uric acid- $p < 0.001$ and, malondialdehyde- $p < 0.001$ in non-alcoholic fatty liver disease, with diabetes mellitus and obesity as compared to healthy controls, and also found the positive correlation of serum ferritin, uric acid, and malondialdehyde. We found that the significantly increased levels of serum ferritin, uric acid and, malondaildehyde as a useful marker of increasing risk for non-alcoholic fatty liver disease.

P190

Study of Cystatin-C Level as a Predictive Marker in Renal Disease

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This study was undertaken to evaluate clinical usefulness of cystatin C levels of serum in predicting renal impairment in normalbuminuric with type 2 diabetes mellitus (T2DM). Diabetic nephropathy is the most common cause of microvascular chronic complication of T2DM which is associated with considerable morbidity and mortality. Chronic kidney diseases (CKD) may result from

like Diabetes Mellitus (34%), hypertension (29%), glomerulonephritis (14%) and others (23%). The present study was undertaken to explore the possibility of the serum cystatin C level as marker of early renal impairment in T2DM patient. The present study carried out estimation of cystatin C level and cystatin C estimated eGFR. The present study carried out estimation of lipid profile. The study was conducted in 75 patients of type 2 diabetes mellitus and 75 healthy individuals were included as controls in this study. After 12 hrs, fasting 8 ml. venous blood sample was collected and allowed to clot for half an hour and serum was separated. Lipid profile and cystatin C level of serum were measured by using commercially available kit of auto analyzer. The level of Serum cystatin C was significantly increased in T2DM Patients as compared to controls. In T2DM patients HDL cholesterol significantly decreased ($P < 0.001$), while other parameters of lipid profile were significantly increased ($P < 0.001$) as compared to control group. Present study suggests that cystatin C measurement in serum is a useful, practical, non-invasive tool for the evaluation of renal involvement in the course of diabetes, especially in normoalbuminuric patients and study also shows significant lipoprotein abnormalities in T2DM patients when compared to controls.

P191

To Study the Activity of Lecithin: Cholesterol Acyl-Transferase in Alcoholic Liver Cirrhosis

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Alcoholic liver cirrhosis is the most common complication of ethanol abuse. About 8% to 20% of heavy drinkers have alcoholic cirrhosis. Alcoholism produces alteration in lipoprotein metabolism inducing liver steatosis and necrosis. Lecithin-cholesterol acyltransferase (LCAT) is a plasma enzyme that esterifies cholesterol and increases high-density lipoprotein cholesterol (HDL-C). LCAT promotes reverse cholesterol transport (RCT) and maintains a free cholesterol gradient between HDL and peripheral tissues. We studied the activity of LCAT in alcoholic liver cirrhosis and normal healthy control groups. A cross sectional study which included 100 males (50 alcoholic liver cirrhosis patients and 50 healthy controls) between age group of 25–55 years from BIMS Hospital, Belagavi. LCAT activity was assessed by measuring the difference between esterified and free cholesterol by digitonin precipitation method. HDL cholesterol level was measured by CHOD-POD method. LCAT activity and HDL cholesterol values were decreased significantly in alcoholic liver cirrhosis patients when compared to controls ($p < 0.05$). The reduced LCAT activity and HDL cholesterol may be associated with a reduction in reverse cholesterol transport (RCT) and may contribute to the development of atherosclerosis in alcoholic liver cirrhosis patients.

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Comparative Study of the Status of Oxidative Stress Markers, Antioxidant Enzymes in Alcoholic Hepatitis Patients

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The exact pro-oxidant and antioxidant status in alcoholic hepatitis is still not clear. The present study was conducted in Department of Biochemistry, RMRI, Bareilly and Santosh medical college and Hospital. 35 alcoholic hepatitis patients were subjected to detailed clinical examination and laboratory investigations and the results were compared with 35 controls. Blood samples were collected for oxidative stress parameters. It was observed that there was a significant increase in activities of SOD, GPX and Catalase activity in patients with alcoholic hepatitis when compared to controls. Results of our study depict higher oxygen free radical production, evidenced by elevated levels of MDA and decreased levels of GSH, ascorbic acid, vitamin-E and Catalase activity, supporting the evidence of oxidative stress in alcoholic hepatitis patients. Increased activities of antioxidant enzymes might be a compensatory regulation of body in response to increased oxidative stress. Decreased concentrations of antioxidant vitamins support the hypothesis that alcoholic hepatitis is an important causative factor in pathogenesis of lipid peroxidation. These data reveal that antioxidant defense mechanisms might be impaired in patients with alcoholic hepatitis. These findings also provide a theoretical basis for development of novel therapeutic strategies, such as antioxidant supplementation.

P193

Role of Procalcitonin in Chronic Kidney Disease Patients Before and After Dialysis

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Chronic kidney disease is a worldwide health problem and hemodialysis is the treatment which is preferably given to the patients who do not undergo renal transplant therapy. Procalcitonin has been described as new marker of inflammation, it has been extensively studied in dialysis patients. It is important to find the significance of Procalcitonin (PCT) in patients of CKD on dialysis. To

find importance of Procalcitonin in CKD patients before and after dialysis. 40 CKD patients were enrolled in the study. Routine investigations were performed using fully auto-analyser. Also, Procalcitonin was checked using i-chroma. PCT levels were significantly high before dialysis 11.84 ± 25.76 ng/ml (Mean ± S.D) as compared to the levels of PCT after dialysis 8.38 ± 22.32 ng/ml. PCT levels were decreased by 29.22% after dialysis. ($p < 0.05$). It was observed that PCT levels increased in patients on chronic hemodialysis and PCT is a possible inflammatory marker in chronic kidney disease patients.

P194

Lipid Profile, Apolipoprotein (A1 and B) and Lipoprotein (a): Predictors of Dyslipidemia and Severity of Liver Damage in Alocoholic Cirrhosis

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Liver plays a central role in lipid metabolism. Our aim was to assess the severity of Liver damage in Alcoholic Cirrhosis by Child Pugh Classification (CPC), Model End Stage Liver Disease (MELD) scores and correlating with lipid levels. We attempted to know the pattern of lipid abnormalities and assessed its role in severity of cirrhosis. In this cross sectional study, 60 patients with alcoholic cirrhosis and 60 age and sex-matched healthy subjects (controls) were studied. Serum triglyceride levels, total cholesterol, HDL, LDL, VLDL, Apo A1, Apo B100 and lipoprotein (a) were measured. CPC and MELD Scores were calculated for each patient as an index for the extent of liver damage. There was a significant decrease in serum triglyceride, total, LDL and HDL cholesterol levels in cirrhotic compared to the control group ($p < 0.05$). Apo A1 and Apo B100 levels in cases significantly reduced when compared to control group ($p < 0.005$) in regard to CPC scores and MELD scores. Lipoprotein (a) were significantly reduced ($p < 0.05$) in Child C cirrhosis. Serum Total Cholesterol, LDL, HDL and VLDL were significantly lower in cirrhotics. Apo A1 and Apo B100 level was significantly reduced with severity of liver damage. Lp (a) levels was significantly reduced in Child C cirrhosis. Hypolipidemia may be due to decreased synthesis of Apolipoproteins, reduced HDLc and decrease production of LCAT. Studies have shown that severe sepsis is frequently associated with hypocholesterolemia in cirrhosis and low level of Apo A1. In conclusion, dyslipidemia exists in patients with cirrhosis.

P195

Study of Enzymes (SGPT, SGOT and GGT) and Lipid Profile in Non-alcoholic Fatty Liver Disease

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NAFLD is one of the most common causes of fatty liver occurring when fat is deposited in the liver, due to causes other than excessive alcohol use. It is one of the most common liver disorders in developed countries. NASH is the extreme form of NAFLD, and is regarded as a major cause of cirrhosis of the liver of unknown. In our study BMI and USG play a very important role to diagnose a patient. We estimate lipid profile, enzyme (SGOT, SGPT, and GGT in NAFLD patients. The present work was done in department of medical biochemistry in co-ordination with department of medicine, Gandhi medical college, Bhopal. The study was carried out 100 person (50 patients and 50 healthy person). The enzymes SGPT and SGOT Are estimated by UV-kinetic method and the GGT are estimated by end point reaction method, the lipid profile and total cholesterol are estimated by CHOD-POD method. TG are estimated by GPO-POD method. In our study the total no of patients are 50 as compared to control group. In our study the level of SGPT, SGOT and GGT are significantly increased as compared to control group but in some cases GGT levels are increased and decreased. The SGPT levels are 60.75_ + 8.85 significantly higher as compare to control group 27.71_ + 6.87. The SGOT levels are 67.54_ + 9.0 significantly higher as compared to 25.86_ + 7.84. The level of lipid profile also increases as compared to control group. NAFLD is a multi-factorial disorder with contribution of a variety of genetic and environmental factors. NAFLD may progress to the irreversible steatohepatitis (NASH) and further cirrhosis or hepatocellular carcinoma.

P196

Maintenance Hemodialysis and its Effect on Lipid Profile of CRF Patients

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Chronic renal failure (CRF) is associated with profound lipid disorders, resulting from dysregulation of high-density lipoprotein (HDL) and triglyceride-rich lipoprotein metabolism. This study was aimed to see the effect of hemodialysis on lipid profile of CRF patients. This was a cross-sectional study, done in the department of Biochemistry, Pt. B.D. Sharma, PGIMS, Rohtak. Thirty patients diagnosed with chronic renal failure on maintenance hemodialysis and thirty age and sex matched healthy controls were enrolled in the study after taking informed consent. Levels of blood urea, serum creatinine and lipid profile were estimated in control group as well as in pre and post hemodialysis samples of CRF patients. Mean serum HDL-C level (30.6 ± 6.78 mg %) was found to be significantly reduced ($p < 0.05$) in CRF patients as compared to that of controls (47.56 ± 4.98 mg %). The mean serum level of total cholesterol (210.7 ± 25.06 mg %), LDL-C (123.7 ± 16.40 mg %) and TG (138.83 ± 34.23 mg %) were significantly elevated ($p < 0.05$) in CRF patients as compared to controls but after hemodialysis these parameters were significantly reduced ($p < 0.05$) as compared to pre hemodialysis. Level of HDL-C was also elevated after hemodialysis ($p < 0.05$). Reduced TC/HDL-C and LDL-C/HDL-C of the hemodialysed patients suggest the beneficial role of hemodialysis on lowering the cardiovascular risk factors in chronic renal failure patients.

P197

Association of RAGE Gene Polymorphisms with Serum AGE Level, Urinary Albumin /Creatinine Ratio and Efficacy of Angiotensin Converting Enzyme Inhibitor Therapy in Type 2 Diabetic Patients with Nephropathy

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Diabetic nephropathy (DN) is a clinical syndrome with persistent micro/ macroalbuminuria and is the major micro-vascular complication of type 2 diabetes mellitus (T2DM). Hyperglycemia mediated advanced glycation end products (AGE) formation is the one of the major factor leading to diabetic vascular complications. Sustained interaction of advanced glycation end products with their receptor RAGE and subsequent signaling plays an important role in the development of diabetic vascular complications. Therefore, Genetic variation of RAGE gene may be associated with the micro-vascular complication in type 2 diabetes mellitus. The present study was designed to explore the association of RAGE gene polymorphisms viz; -374 T/A, -429T/C and Gly82Ser with serum AGE level, urinary albumin/creatinine ratio (ACR) and efficacy of angiotensin converting enzyme (ACE) inhibitor therapy in type 2 diabetic patients with nephropathy. The study subjects comprised of clinically diagnosed type 2 diabetes mellitus patients ($n = 200$) with evidence of persistent micro-albuminuria (ACR; 30 - 300 mg/g creatinine) or overt albuminuria (ACR < 300 mg/g creatinine) tested on two separate occasions. These patients were treated with Ramipril (5 mg to 20 mg) for 12 months. 5 ml blood samples were collected for biochemical analysis and genotyping. Genotyping of RAGE variants were assessed by polymerase chain reaction-restriction fragment length polymorphism. Serum creatinine and urine creatinine were measured by alkaline picrate Jaffeés kinetic method. Urine microalbumin was estimated by nephelometer. Serum AGE level was determined by spectro-fluorometry at emission maximum (440 nm) and excitation maximum (350 nm) and fluorescence intensity was expressed in arbitrary unit (AU). ACE inhibitor treatment produced significant fall in urinary ACR and significant reduction of serum AGE level during 12 months follow up period as compared to baseline value. Mutant variants of RAGE gene were significantly associated with increased levels of serum AGE, more proteinuria and worse renal function and degree of response to ACE inhibitor varies in patients with their corresponding genotypes. ACE inhibitor treatment has shown antiproteinuric and AGE lowering effect indicating its effect towards lowering of micro-vascular compilation in diabetic patients. RAGE gene polymorphisms are likely to be associated with progression of nephropathy and response to treatment with ACE inhibitor in DN patients.

P198

Role of RAAS Gene Polymorphisms in the Efficacy of ACE Inhibitor Mediated Reduction in Proteinuria in Type 2 Diabetic Patients with Nephropathy

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Diabetic nephropathy (DN) is a clinical syndrome with persistent micro/ macroalbuminuria and is the leading cause of end stage renal disease (ESRD) worldwide. It affects approximately 40% of diabetic patients. Angiotensin converting enzyme (ACE) gene plays an important role in the pathogenesis of DN. ACE inhibitors (ACEI) are commonly prescribed as antiproteinuric therapy in diabetic nephropathy patients. However, the antiproteinuric response of ACE inhibitor therapy is not uniform in all patients. The polymorphisms of enzymes/proteins of the renin-angiotensin-aldosterone system (RAAS) may be related to antiproteinuric response of ACE inhibitor therapy. The aim of this study is to evaluate the role of angiotensin converting enzyme (ACE I/D), angiotensinogen (A235T) and angiotensin II type 1 receptor (A1166C) gene polymorphisms on the efficacy of ACE inhibitor therapy. In the present study, type 2 diabetic patients with nephropathy ($n = 200$) were recruited and treated with Ramipril (initial dose was 5 mg/day) and followed-up at 12 months for clinical and biochemical analysis. Urine albumin was estimated by nephelometer and creatinine by Jaffe's kinetic method. The result was expressed as urine albumin/creatinine ratio (ACR). Genotyping study was performed by using primer specific PCR and PCR-RFLP techniques. All DN patients benefited with respect to proteinuria on Ramipril therapy after 12 months follow up. On subdividing the patients as microalbuminuric (ACR > 30–<300 mg/gcr) and macroalbuminuric (ACR > 300 mg/gcr), it was observed that the patients response to Ramipril therapy depends on the status of albuminuria and corresponding genotypes present in them.

P199

Assesment of Status of Renal, Hepatic Disorders and Diabetic Status in Geriatric Age Group Admitted in Medical wards Govt. General Hospital, Vijaywada

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Health assessment in geriatric age group is necessary to help them take interventions to prevent certain ailments and to change their life style for a better health and improve quality of life.

The present study was aimed to Perform Renal function tests, Liver function tests, Lipid profile and RBS and to assess their health status and guide them to seek clinician's advice accordingly. Present study was carried out in a home for old age, Vijayawada. Total no. of subjects were 75. Sample collected is fasting blood sample. Tests conducted 1) Urea 2) creatinine 3) Bilurubin, 4) AST 5) ALT 6) ALP 7) Total Cholesterol 8) HDL cholesterol 9) Triglycerides 10) Random Blood Sugar. Investigations were conducted using kit methods in semi auto analyzer. Permission was given to conduct the tests in the home for old age on 19th Sep. 2015. My aim is to group the disorders and calculate the percentage as follows percentage with Hepatic disorders, percentage with Renal disorders, percentage with Diabetes Mellitus, percentage with Hyper Cholesterolemia, percentage with No ailments and miscellaneous ailments data will be presented in the conference. The study population is advised accordingly to prevent certain ailments by changing life style and to take interventions towards better health and improve quality of life.

P200

Relationship of Vitamin B_{12}, Folic Acid Levels in Patients with Chronic Liver Disease

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Liver is a vital organ which performs functions relating to digestion, metabolism, detoxification & many more. Whenever the damage to the liver continues, parenchyma deteriorates & fibrosis leading on to cirrhosis is ultimate. Vitamin B12 and Folic acid serves as a coenzyme and are involved in the synthesis of proteins that are essential for the integrity and function of cell membranes. In this context Folic acid along with Vitamin B12 are vital parameters which can indicate the initialization of underlying pathology. The aim of the study was to estimate the serum levels of Vitamin B12, Folic acid in chronic liver disease patients and to compare the serum levels of Vitamin B12, Folic acid between chronic liver disease patients and healthy control population. This study was conducted in the Department of Biochemistry and Medicine, G.G.S Medical College, Faridkot. It was a case control study. 40 cases (25 with alcohol etiology and 15 as non-alcoholic) of chronic liver disease were included in this study with equal number of healthy people who served as controls. The patients with chronic liver disease of varied etiology were studied. Mean value of serum folate levels in CLD patients was 6.802 ± 3.42 ng/ml and exhibited lower value than controls. Serum vitamin B12 which was taken as second parameter exhibit high values of 1291 ± 612 pg/ml than controls. The levels of vitamin B12 and folic acid were deranged in chronic liver disease. Vitamin B12 levels increased due to necrosis in the liver. Decreased levels of folic acid were due to nutritional deficiency or toxic effect of alcohol. The levels may be of use in differentiating alcohol liver disease from non-alcohol liver disease.

P201

Study on the Enteroaggregative *Escherichia coli* Induced Apoptosis in Cultured Human Intestinal Epithelial Cells

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Enteroaggregative *Escherichia coli* (EAEC) is emerging as an important enteric pathogen causing diarrheal diseases in multiple epidemiological and clinical settings. However, understanding of the pathogenesis of this organism is still suboptimal. Epithelial cell apoptosis is defined as a new category of intestinal epithelial cell response to enteric infection. However, there is no report regarding EAEC induced apoptosis in intestinal epithelial cells. The study was aimed to assess the EAEC induced apoptosis in cultured human intestinal epithelial cellsand the role of membrane proteins of this organism in this process. INT-407 (human small intestinal epithelial cell line) and HCT-15 (human colon carcinoma cell line) cells were infected with an invasive, non-invasive prototype and plasmid cured variant of EAEC separately. Apoptosis was assessed in EAEC infected both the cell lines as well as cells cultured in presence of membrane proteins of EAEC-T8, EAEC-pT8 & plasmid-borne EAEC-T8membrane proteins by dual staining with Annexin-V-FLOUS & PI, internucleosomal cleavage of host cell DNA, alteration in the mitochondrial membrane potential and expression analysis of different parameters of apoptotic pathways. Invasive wild type EAEC strain revealed maximum extent of apoptosis in case of both the cell lines as compared to EAEC-042and EAEC-pT8indicating the importance of invasion and plasmid in EAEC induced apoptosis. The plasmid borne membrane proteins of EAEC were found to have maximum involvement to this process. This study has undoubtedly improved the understanding of pathogenesis caused by enteric pathogen.

P202

Role of an Isolated Compound from *Ageratum conyzoides* L. in Indomethacin Induced Gastric Ulcer in Albino Rats

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Reports on clinical evaluation of presently available anti peptic ulcer drugs show that there are incidences of relapses, adverse

effects and danger of drug interactions during ulcer therapy. Hence, search for an ideal antiulcer drug continues which may afford better protection and decrease the incidence of relapse. The aim of this study was to see the role of a compound, isolated by us from *Ageratum conyzoides* L., in indomethacin induced gastric ulcer in albino rats. Gastric ulcers were induced in 10 h fasted rats by oral administration of indomethacin (10 mg/kg) in two doses at an interval of 15 hour. Compound isolated from *Ageratum conyzoides* L. was given in doses (50 mg/kg and 100 mg/kg) in another two groups of rats orally 30 minutes prior to each dose of indomethacin. Ranitidine (50 mg/kg p.o.) was used in another set of experiment for comparison. 4 h after administration of last dose of indomethacin, rats were sacrificed. Stomachs were taken out and examined for ulcers. Anti-oxidant enzymes like superoxide dismutase, catalase, glutathione, and glutathione peroxidase were measured in gastric mucosa. Compound isolated from *Ageratum conyzoides* L. significantly reduced ulcer index in rats induced by indomethacin and enhanced the activity of anti-oxidant enzymes. Results were comparable to that of ranitidine, a standard anti-ulcer drug. Compound isolated from *Ageratum conyzoides* L had anti gastric ulcer activity in indomethacin induced gastric ulcer in albino rats and the activity was mediated through anti-oxidant defense mechanism.

P203

Polymorphisms in the Cytochrome P450 (*CYP1A2*) in Chronic Kidney Disease of Unknown Etiology Patients and Controls and its Influence on Organochlorine Pesticide (OCP) Level

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Chronic kidney disease (CKDu) of unknown etiology represents about 16% of CKD patients in Indian subcontinents and 10% worldwide. The etiology of CKDu remains unclear though epidemiological studies indicate the involvement of environmental toxins. Organochlorine pesticide (OCPs), are thought to be an important environmental causal factor, as the presence of OCPs have been detected in the blood of general population in India. Polymorphism in Cytochrome P450 CYP1A2 gene has been associated with several disorders like cardiovascular disease. It is possible that polymorphism in this gene may also play an important role in causing CKDu. In this study attempts have been made to evaluate the effect of CYP1A21C/CYP1A21F polymorphism in CKDu patients and to find out the blood levels of OCPs in same. In this study we have assessed 200 CKDu patients and 200 age-sex matched healthy controls. The blood OCP levels were analysed by Gas Chromatography. CYP1A21C/CYP1A21F genotyping were carried out by restriction fragment length polymorphism (RFLP). Blood levels of aldrin, p,p-DDE, HCH and total pesticides were significantly higher in CKDu patients and negatively correlated with eGFR. It is also evident from the results that polymorphism in CYP1A21C is significantly associated with the disease whereas the polymorphism in CYP1A21F is not associated with the same.

P204

Increased Level of Organochlorine Pesticides in Chronic Kidney Disease Patients of Unknown Etiology: Role of *CYP1A1* Gene Polymorphism

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Chronic kidney disease (CKDu) of unknown etiology represents around 16% of CKD patients in Indian subcontinent and10% worldwide. The etiology of CKDu of unknown etiology remains unclear though epidemiological studies indicate the involvement of the environmental toxins. Organochlorine pesticides (OCPs) have been detected in general population in India. It is possible that polymorphism of xenobiotic metabolizing enzymes (XMEs) may play an important role in the pathogenesis of the disease process. The present study has been designed to find whether a blood level of OCPs is associated with CKD patients of unknown etiology and to evaluate the consequence of *CYP1A1* gene polymorphism on the same. Two groups of study subjects were recruited for this study. Group 1:- Healthy subjects (n = 200), Group2:- Patients with chronic kidney disease (n = 200) of unknown etiology (CKDu). Blood OCPs levels of all study groups were analyzed by gas chromatography.*CYP1A1*2A* and **2C* polymorphisms were studied by PCR-RFLP and allele specific-PCR respectively. Blood levels of α-HCH, γ-HCH, α-endosulfan, aldrin, p'p'-DDE and total pesticides were significantly higher in CKD patients as compared to healthy subjects and negatively correlated with eGFR. *CYP1A1*2A*(OR = 1.62, p = 0.03) and *CYP1A1*2C* (OR = 1.66, p = 0.01) polymorphisms were found to be significantly associated with the development of CKD. Polymorphism of XMEs not only increased accumulation of pesticides but also aggravates kidney dysfunction as evident from significant decrease in eGFR.

P205

Serum Creatinine can be Replaced – Myth or Fact?

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Acute kidney Injury (AKI) is assessed by an increase in serum creatinine. Several injury markers like urinary Neutrophil gelatinase associated lipocalin (uNGAL) and Calprotectin (uCal) have been recently reported. However their data amongst Indians is scarce. We conducted two studies to assess the efficacy of uNGAL and uCal as markers of AKI in patients undergoing Coronary artery bypass graft surgery (CABG). For uNGAL study urine was collected on admission and 3 hours after CABG from 40 adults. Chemiluminescent

Magnetic Immunoassay (CMIA) was used for uNGAL estimation; its fold increase after surgery was calculated for each patient. In another study urine for Calprotectin estimation by ELISA was collected after 24 hours of CABG (n = 42). The results were compared with healthy controls (n = 20). Both study results were sorted based on evidence of AKI as per AKIN criteria. In patients having AKI (n = 23) median fold increase and inter quartile range (IQR) of uNGAL post-CABG was 2.2 (1.16–4.26) while in non-AKI patients (n = 17) it was 1.85 (0.98–3.3). Median & IQR calprotectin in controls was 121 μg/g creat (50.2–498 μg/g), in AKI patients (n = 23) was 1104 μg/g creat (354 –1534 μg/g) while in non AKI patients (n = 19) was 1055 μg/g creat (447–2016 μg/g). Both uNGAL and uCal showed overlapping results in AKI and non-AKI patients However uCal levels were higher in patients than controls. The results suggest that both uNGAL and uCal fail to detect AKI and hence serum creatinine still remains to be the most reliable marker of AKI.

P206

Effect of Antitubercular Therapy on Serum Lipid Profile in Tuberculosis Patients and Its Correlation with Conventional Markers of DILI

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Among the various factors predisposing to antitubercular drug (ATD) induced hepatotoxicity, both genetic and acquired, are well delineated, but little is known about the cellular and biochemical mechanisms. Impaired lipid metabolism oftenly results in steatosis and provides substrate for lipid peroxidation. It triggers inflammation, apoptosis, and progression to steatohepatitis and cirrhosis. Change in serum lipids may be one of the contributing factors towards such antitubercular drugs induced liver injury. The present study was designed to examine the ATD induced lipid changes in serum of tuberculosis patients and correlate it with currently used parameters of DILI. Study population was confirmed cases of tuberculosis patients on antituberculosis medications. Control group comprised of two sets of individuals: Set I - 25 apparently healthy individuals and Set II-25 newly diagnosed tubercular patients who had not received any ATD therapy. For Enzymes and lipid fractions samples were analysed on Random access autoanalyzer (Modular-P) using standard kits. Data was analyzed by ANOVA followed by multiple comparisons -Dunnett's procedure and Newman–Keul's procedure. Serum ALT levels in control groups were 17.6 ± 6.1U/L and 17.8 ± 7.2 U/L respectively in Set I and Set II. On measuring serum ALT levels in study population three groups of patients were found: Group I ALT = 15.3 ± 5.2U/L, Group II ALT = 36.2 ± 8.5U/L and Group III ALT = 136.2 ± 85.7U/L respectively. Total cholesterol in Set I and Set II control groups were 133.36 ± 18.31 mg/dL and 131.6 ± 20.2 mg/dL respectively. Administration of ATD therapy resulted in increased serum total cholesterol levels (148.2 ± 36.25 mg/dL,168.05 ± 46.4 mg/dL and 182.8 ± 44.29 mg/dL respectively in Group I, II and III) which were significantly higher in Group III as compared to control group. Changes in serum lipids are likely to be involved in the pathogenesis of ATD therapy induced hepatoxicity in case of tuberculosis patients.

P207

Chronomics of Serum and Urinary Urate in Normals and Renal Stone Formers

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Chronomis, as part of a broader time structure (chronome) of serum and urinary urate may communicate to prevention and management of urolithiasis. Fifty healthy volunteers (age: 20 to 40 years) and 100 renal stone formers, admitted in Surgical wards of Shri Mahant Indiresh Hospital, Shri Guru Ram Rai Institute of Medical and Health Sciences, of similar age group with diurnal activity from 06:30 to about 22:00 and nocturnal rest were included to study chronomics of serum and urinary urate. Blood samples were collected every 6 hours for 24-hour period under standardized conditions beginning at 06:00 to 12:00, 12:00 to 18:00 and 00:00 to 06:00. Each subject was asked to collect all the urine voided in a given subspan in a sterile plastic bottle. Serum and urinary uric acid was measured spectrophometrically. A marked circadian variation in uric acid concentration was recorded in both the groups with peaks around 14:00 and 13:00 hours respectively. Similarly, one-way ANOVA also confirmed a significant circadian rhythm between the two groups ($p < 0.05$). A significant circadian rhythm was also observed in urinary uric acid levels in healthy volunteers and renal stone formers with peak around noon. However, serum uric acid was found to be elevated at all time points during 24-hour period in stone formers in comparison to healthy subjects. The excretory pattern, however, did not exhibit any significant difference in uric acid levels between the two groups although the excretion was higher in stone formers as compared to healthy controls. Maximum uric acid excretion around midday might be the peak hour for maximum crystallization of stones containing an admixture of urate in stone formers; which could be clinically important in further management in minimizing the risk of crystal growth (urate admixture) in renal tubules.

P208

Irisin and Visfatin Levels in Diabetic Nephropathy

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Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) worldwide, and it is estimated that 20% of type 2 diabetic patients reach ESRD. Visfatin plays an important role in renal injury associated with insulin resistance and associated with the

progression of diabetic nephropathy. Irisin has been identified as a novel adipomyokine that drives brown-fat-like conversion of white adipose tissue and thermo genesis. There is scarcity of data assessing irisin level in diabetic nephropathy. The aim of the study was to assess the levels of Visfatin and Irisin in patients with diabetic nephropathy in comparison with diabetic without nephropathy and to identify the association of Visfatin and Irisin with severity of diabetic nephropathy. 40 diabetic nephropathy subjects as cases and 40 diabetic without nephropathy subjects as controls based on fundoscopy and ultrasonography abdomen were enrolled in the study. Serum levels of Visfatin and Irisin were estimated by ELISA and Fasting Glucose, Fasting Lipid Profile and Creatinine were estimated in both the groups. Disease severity was assessed by eGFR. Serum levels of Irisin and Visfatin were significantly increased in diabetic nephropathy compared with diabetic without nephropathy. Elevated serum Irisin and Visfatin levels were associated with disease severity in diabetic nephropathy.

P209

Allergic Profile in Patients of Bronchial Asthma

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Allergic diseases are amongst the most common chronic disorders worldwide. Bioparticulate matter like pollen grains, fungal spores, insect debris, house dust mites, animal dander, chemicals, foods, etc. have been implicated as cause allergic symptoms. Allergy has been stated as an important cause of bronchial asthma, but data lacks on the specific IgE which may be a useful marker of allergic response. The study was aimed to study the allergic profile in patients of bronchial asthma by determination of specific IgE for common inhalation antigen. This cross sectional study was done on 70 patients of bronchial asthma diagnosed by the GOLD criteria.5 ml blood sample was taken to do enzyme immunoassay for semi quantitative determination of specific IgE for 09 common allergens which included grass mix, weeds mix, animal mix, epithelia mix, corn, dermatophagoides, aspergillus, eucalyptus and mould mix. Presence and absence of the allergens was tested and their percentage in the study population studied. Out of the 9 allergens tested in the study population of 70 bronchial astma patients, dermatophagoides was found to be the most prevalent allergen (18.57%). The other allergens in decreasing percentage of prevalence were grass mix, corn and eucalyptus, weed and aspergillus. None of the samples were found positive for dust mite, animal mix and mould mix. Specific IgE would be useful for both diagnosis and planning treatment strategies in asthma. Quality of life can certainly be improved in such patients by decreasing the frequency and severity of allergic attacks.

P210

Proteomic Biomarker for Early Detection of ATD Induced Liver Injury

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Incidence of ATD induced hepatotoxicity, varies between 2% and 28% in Indian population. Standard biomarkers for drug induced liver injury (DILI) are a combination of four tests, including serum ALT, AST, ALPand TBL concentration. Elevation of ALT > 3 folds and TBL > 2 folds than normal range, indicates liver injury. However, the increase in serum ALT activity level has also been associated with other organ toxicities, thus, indicating enzyme specificity beyond liver. Asymptomatic transaminase elevations occur in patients during standard antitubercular regimens which usually resolve. Thus, more sensitive and specific diagnostic biomarker is required for early diagnosis of DILI. So in our study a proteomics based approach has been used to identify changes in the serum proteomic profile of TB patients who are on anti-TB therapy and having DILI due to ATDs. Blood samples were collected from 10 patients under each group: healthy, newly diagnosed TB patients not on ATDs, TB patients on ATDs (ALT/AST < 2 fold), TB patients on ATDs (ALT/AST level >2 fold). Serum proteome was done using two dimensional gel electrophoresis. 2D gel electrophoresis and software analysis of serum proteins of tuberculosis patients with and without hepatotoxicity showed some up and down regulated proteins as compared to healthy individuals Further trypsin digestion and MALDI MS analysis of these proteins revealed identification of proteins involved in the important metabolic processes. Work is in progress to validate these proteins further and elucidate their role in drug induced liver injury. Differentially expressed proteins in DILI can be used to novel biomarkers for early ATD induced liver injury

P211

Evaluation of 25-Hydroxy Vitamin D in Ultrasonographically Diagnosed Cases of NAFLD

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Hypovitaminosis D has been recently recognized as a worldwide epidemic. Vitamin D exerts significant metabolic activities, comprising free fatty acids (FFA) flux regulation from the periphery

to the liver. Its deficiency may promote fat deposition into the hepatocytes. We aimed this study to evaluate serum 25-Hydroxy Vitamin D levels in 60 ultrasonographically diagnosed subjects of NAFLD and 20 controls. The study group comprised of 60 ultrasonographically diagnosed patients. The control group comprised of 20 healthy age and gender matched individuals. Alcoholic subjects, patients with infectious hepatitis and autoimmune hepatitis were excluded. Estimation of Vitamin D was done by Enzyme-linked immunoassay (ELISA). The mean age of case group was 48.7 ± 11.1 years and control group was 48.25 ± 9.68 years. The mean value of 25 hydroxy Vitamin D in study group and control group were 12.2 ± 6.8 ng/ml and 30.1 ± 17.9 ng/ml, respectively.(Normal Range of Vitamin D: 30–74 ng/ml). The decrease in Vit D level in study group versus control group is statistically significant ($p < 0.001$. Our study demonstrates a strong association between low 25-(OH) vitamin D levels and NAFLD in a population where mean age of study group was 48.7 ± 11.1 years. Our study suggests a significant contribution of hypovitaminosis D in the pathogenesis of liver steatosis.

P212

To Study Serum Sodium and Potassium Level in Patients with Alcoholic Liver Disease Attending JMCH

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Hyponatremia is a common abnormal finding in approximately 57% of hospitalized patients with chronic liver disease and in 40% of outpatients with liver disease. Chronic alcoholic patients experience low blood concentrations of key electrolytes as well as potentially severe alterations in the body's acid-base balance.. Aim of the study is to evaluate serum sodium and potassium level in patients with alcoholic liver disease attending JMCH. The study design is hospital based case control study. For the study 40 no of cases are selected on the basis of clinical history, and 40 no of apparently healthy age and sex matched individuals have been taken up from normal population as control group. LFT and serum sodium and potassium will be done on vitros 250 autoanalyser based on principle of reflectance spectroscopy. After evaluation hyponatremia is found among the cases and statistically significant. But the serum potassium level is though mildly decreased, no statistically significant results were found.

P213

Interleukin-1 Polymorphism and Expression in Hepatitis B Virus Mediated Disease Outcome in India

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Hepatitis B Virus (HBV) infection is a primary factor of hepatocellular carcinoma (HCC), the fifth most frequent cancer, worldwide. Interleukin (IL)-1 cytokine is considered to be a key mediator in HBV linked disease progression. The study was aimed to study the distribution of *IL-1B* (-511C>T) and *IL-1RN*(VNTR) polymorphism and haplotypes and their association with HBV-HCC risk. Also, to analyze the expression and levels of IL-1B in different categories of HBV patients, 406 subjects (153 healthy controls, 67 inactive HBV-carriers, 65 patients with chronic-active HBV, 62 HBV cirrhotic and 59 subjects with HBV-HCC) were enrolled in the study. Polymerase chain reaction (PCR)–restriction fragment length polymorphism was carried out to study the genotype frequencies. IL-1B expression was evaluated by real-time reverse transcriptase (RT)–PCR analysis by using sequence-specific primers. IL-1B levels in peripheral blood mononuclear cells (PBMCs) were estimated using an enzyme-linked immunosorbent assay (ELISA). The study revealed no significant association of *IL-1B* (-511) CT and TT genotypes with HCC development. However, the IL-1 haplotypes 2 and 3 were found to be significant protective factors for hepatitis and subsequent HCC development, among controls while haplotype 4 shared a significant negative association with hepatitis only. A significant positive association of the *IL-1RN* (VNTR) 1/2 genotype with HCC development was observed among controls and carriers. Besides, 2/2 genotypes acted as a potential risk factor for hepatitis and subsequent cirrhosis development, among the same groups. Moreover, proinflammatory IL-1B levels significantly and steadily elevated with the disease progression to HCC, as compared to controls. These preliminary findings indicate a key role of IL-1 in the HBV-mediated disease chronicity, in the Indian population.

P214

Malarial Hepatopathy-Experience at Tertiary Care Centre of North India

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Jaundice is commonly seen in severe malaria (approx. 2.5% patients) but hepatitis is unusual. Hepatocellular dysfunction varies from mild abnormalities in liver function tests (LFTs) to hepatic failure. The aim of the study was to study the clinical, biochemical profile, complications and outcome in confirmed Plasmodium falciparum malaria cases with hepatopathy. This retrospective study was carried out in a tertiary care hospital in NorthIndia by reviewing slide confirmed case records of P. falciparum malaria with biochemical evidence of hepatic dysfunction, admitted between 1/10/2012 and 1/10/ 2013. A total of 13 patients (all male) with mean age 43.07 years, mean duration of fever prior to hospitalization 6.5 days, were included. Fever persisted in all patients after the onset of jaundice. Encephalopathy was present in 38.5% (5) of patients. Hepatosplenomegaly, icterus and anaemia (Hb < 10 gm %) were found in 84.61%, 92.30% and 84.61% respectively. Predominant (> 50%) conjugated hyperbilirubinaemia was found in all the patients, with mean total bilirubin level of 21.06 mg /dL (1.5–54). Bilirubin concentration of >10 mg/dL was associated with renal failure (serum creatinine >2.0 mg/ dl) in 77.8% (7) cases. Mean AST, ALT and ALP levels were 164.84 IU/L (38–665), 75 IU/L (43–160) and 132.46 IU/L. AST was >3 upper limit of normal in 53.84% cases as compared to ALT (15.38%). Malarial hepatitis is a serious complication in patients with P. falciparum malaria. Renal dysfunction is more common in those with hyperbilirubinaemia. Whether this is cause or effect, is difficult to hypothesize.

P215

Cytokines in Chronic Obstructive Pulmonary Disease: An Interim Analysis

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Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of morbidity and mortality and results in an economic and social burden worldwide. The COPD is a common preventable and treatable disease, characterized by persistent airflow limitation, usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. In this study, the role of inflammatory markers, Interleukin–6 (IL–6), Tumor Necrosis Factor Alpha (TNF–α), and interleukin-IL-1 beta (IL-1 β) in serum sample were investigated by ELISA method. A total of 164 COPD patients, 33 nonsmokers and 131 smokers were recruited. The COPD was confirmed by post-bronchodilator level (FEV1/FVC < 0.70). Data was analyzed by Mann-Whitney U test and Spearman rank order correlation analyses. The mean (± SD) levels of (pg/ml) of TNF–α (55.54 ± 37.80 vs. 72.40 ± 43.17, U = 1660.0, p = 0.040), IL-6 (56.76 ± 31.30 vs. 114.48 ± 105.79, U = 1456.0, p = 0.004) and IL-1 β (3.72 ± 3.85 vs. 6.93 ± 6.39, U = 1375.0, p = 0.001) were significantly different and higher in smokers as compared to non smokers. Further, the inflammatory markers, especially IL-6 and IL-1 β both well correlated ($p < 0.05$) with the COPD severity. Study concluded that inflammatory markers play an important role in COPD and smokers are at high risk.

P216

Evaluation of Vitamin D Status in Indian Children with Osteoarticular Tuberculosis

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Tubercular patients have been demonstrated to have lower serum levels of vitamin D. Such data is not established for paediatric patients with osteoarticular tubercular disease. We aimed to determine serum vitamin D status in paediatric cases with osteoarticular tuberculosis in a developing country and whetherthis was affected by gender, age or site of TB. The values of serum vitamin D were estimated in 100 children with osteoarticular tuberculosis. The patients were divided into 3 groups based on gender, age and site to assess variations of vitamin D levels. The mean vitamin D levels were 42.165 nmol/L. There were no statistical difference between mean vitamin D levels in boys and girls (p value = 0.6143); age groups <5 years and 5–12 years (p value = 0.3857) or spinal and non spinal osteoarticular 44 groups (p value = 0.8350). Hypovitaminosis D was associated with active osteoarticular TB in children. There was no statistical association between serum vitamin D levels and age, sex or site of infection in osteoarticular infections. Hypovitaminosis D was associated with active osteoarticular TB in children. There was no statistical association between serum vitamin D levels and age, sex or site of infection in osteoarticular infections.

P217

Lipid Peroxidation and Antioxidant Status in Fibrocystic Breast Disease With and Without Sudarshan Kriya Yoga

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Sudarshan kriya yoga (SKY) is a unique breathing process advocated by The Art of Living foundation, Bangalore, India. Yogic breathing exercises decreases lipid peroxidation and improves antioxidant status in blood. This study aims to evaluate lipid peroxidation markers Malondialdehyde (MDA) and nitric oxide (NO) together with total antioxidant capacity (TAC) in fibrocystic breast disease patients. Lipid peroxidation markers i.e. Malondialdehyde (MDA) andnitric oxide (NO) together with total antioxidant capacity (TAC) were evaluated using spectrophotometer (Elico Company) in serum of 30 fibrocystic breast disease patients with routine treatment (control group) and 30 fibrocystic breast disease patients with routine treatment along with sudarshan kriya yoga(SKY) (study group). Blood samples were collected from study group after 7 days of regular practice of sudarshan kriya yoga (SKY). Serum MDA and NO were significantly decreased ($p < 0.001$) with concomitant elevation levels of TAC in study group when compared to control group ($p < 0.001$). Relationship between lipid peroxidation markers i.e. MDA and NO with total antioxidant capacity TAC were inversely related in both groups. Sudarshan kriya yoga practice may improve natural defense system/mechanism and decreases efficacy of oxidative stress in fibrocystic breast disease. However further extensive and long term studies to be done to prove these findings and understand the basic mechanism involved.

P218

High-Density Lipoprotein and Its Role in Infection

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The predominant role of high-density lipoprotein (HDL) has been that of an anti-atherogenic molecule which is attributed to its reverse cholesterol transport. Not much well-known is the role of HDL in innate immunity. Cellulitis is an acute, subacute or chronic inflammation of the dermis and subdermal tissues caused by a bacterial infection. The clinical severity of cellulitis can range from mild infection, to a severe necrotizing infection associated with a high mortality. C-reactive protein (CRP), is a sensitive indicator of inflammatory and infectious disease process in a variety of diseases. However, using CRP as a biomarker in cellulitis has a number of disadvantages like a delayed release and low specificity. Thus this pilot study analyses the role of HDL as a prognostic indicator for estimating the recovery time of patients with celluiltis.

P219

Estimation of Oxidative Stress Level in Individual who have Exposed to Physical Exercise with Dietary Protein Supplementation

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Ceruloplasmin is a copper dependent ferroxidase involved in acute phase reaction with both anti and pro oxidant activity. Oral α-amylase, is one of the most important salivary digestive enzymes which hydrolyse the starch into sugars. Physical exercise induces biochemical changes in the body that modify analytes in saliva among other body fluid. This study analyzed the effect of protein diet on the salivary amylase and Ceruloplasmin in response to physical exercise. Sample consisted of 2 ml unstimulated saliva from 30 voluntary healthy individuals. Control Group had 10 Individuals who do not follow any exercise regimen, Group 1 had 10 individuals undergoing physical training without taking any protein supplements and. Group 2 included 10 individuals undergoing physical training consuming protein supplements. Salivary amylase estimation was done by Di-nitro salicylic acid method and Ceruloplasmin estimation by p-phenol-diammonium dichloride method. Mean salivary Ceruloplasmin in control group and individual undergone physical exercises without taking protein supplement as well as individual undergone physical exercise taking protein supplement were 2.68 ± 0.92, 2.9 ± 1.4 and 3.7 ± 0.71 respectively. The concentration is significant when individuals with protein diet were compared with control ($p < 0.013$). The concentration is not significant between individuals without protein diet were compared with control ($p < 0.677$). Mean salivary Amylase in control group, individual undergone physical exercise without taking protein supplement, individual undergone physical exercise taking protein supplement were 26.2 ± 3.64, 33 ± 3.39 and 37 ± 2.7 respectively. The concentrations were significant when compared with controls ($p < 0.001$). In our study we have shown that exercise may have an impact on oxidative stress level and saliva can be used as non-invasive tool for estimation of oxidative stress.

P220

Effectiveness of Scaling and Root Planning (SRP) on Periodontal Parameters and Systemic Inflammatory Markers in Smokers with Chronic Periodontitis

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Tobacco smoking is one of the main risk factor associated with periodontitis and has long term altered inflammatory, immunological and therapy response. This study comparatively assesses the short term effectiveness of scaling and root planing (SRP) on clinical periodontal parameters and some systemic inflammatory markers between male smokers and non smokers with chronic periodontitis. The study groups comprised of 131 males with sever chronic periodontitis (CAL $\geq$ 5 mm). They were divided into Group I ($n = 51$, mean age: 40.9 ± 4.6) without smoking habits (CP) and Group II ($n = 80$, mean age: 44.1 ± 5.81) with smoking habits (CPSM). The clinical periodontal evaluation was done by measuring gingival index (GI), plaque index (PI), Probing depth (PD) and clinical attachment loss (CAL). The inflammatory markers estimated were interleukins (IL)-6, -10 and C – reactive protein (CRP). SRP was performed on both the study groups with a follow up after 3 months. Smokers with chronic periodontitis showed significantly higher periodontal damage and systemic inflammatory markers compared to non smokers with chronic periodontitis. Post SRP improvement in the mean values (compared to baseline values) was observed in both the groups.

P221

Biotechnology in Disease Diagnosis–Meeting the Needs of a Changing World

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For too long Poor Case Detection has continued to remain a major health care problem, resulting in a huge burden to the patient – (tremendous over treatment/under treatment) and society, (huge implications of cost and drug resistance). Conventional testing methodologies for a variety of infectious and communicable diseases based on so called "Gold Standard" but grossly in adequate platforms have only added to the patient's misfortune. Also the fact that to even take advantage of such existing tests a patient has to necessarily travel long distances to tertiary level centers to give his/her sample for testing and then undergo an agonizing wait for the results of the tests to be known and then for the treatment to get started. Current mdx (molecular diagnostic) platforms using the realtime PCR principles, considered most sensitive and specific, while promising greatly improved delivery of patient care through expedited diagnosis, improved treatment efficacy, have as a matter of fact neither delivered nor met the expectations of "quantum leap in laboratory testing standards". Various complexities and short comings have led to them iniscule use of such a unique technology for the betterment of global health care settings. Not anymore-Leveraging synergies of Bio Micro Electro Mechanical Systems (Bio MEMS) & Polymerase Chain Reaction(PCR), the True lab Real Time micro PCR system, brings real time PCR testing to near patient & Point Of Care (POC) settings for early diagnosis & better patient care & allows the patient–you, to rest assured.

P222

Urinary Schistosomiasis Prevalence, Morbidity Indicators and Associated Risk Factors in Farawa and Koya Endemic Communities, Minjibir, Kano State, Nigeria

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A community-wide cross-sectional study was carried out to determine the prevalence and human risk factors of Schistosoma haematobium infections in Farawa and Koya dam-site communities in Minjibir Local Government Area of Kano State, Northern Nigeria. Urine sedimentation method, urinalysis reagent strip method (Medi Test Combi-9) and questionnaire administration were employed for detection of characteristic parasite egg, morbidity indicators and major risk factors for the infection, respectively. A total of 120 individuals, 60 selected randomly from each community were examined, with overall infection prevalence of 84 (70.0%). The rate of prevalence was slightly higher 44 (73.0%) in Koya than 40 (66.6%) in Farawa community. Increasing infection prevalence was in the order of age groups: 46–55, 26–35, >55, 36–45, 6–15 and 16–25 years. Infection prevalence was gender biased in favour of males with statistical significance ($P < 0.05$), who also manifested with macrohaematuria (75.0%), proteinuria (65.0%) and urine nitrite (15.0%), in Farawa community; macrohaematuria (75.0%), proteinuria (51.9%) and urine nitrite (38.5%) in Koya community. A high rate of macrohaematuria among younger age groups ($\leq$ 35 years) in both communities might be an indication of high infection intensity which portends risk of bladder carcinogenesis at old age. Public enlightenment programme with a view to reducing unprotected exposure to risk factors such as irrigation agriculture, fishing, bathing and domestic water use, and mass chemotherapy targeting younger age groups, will help curb high infection prevalence in the study area.

P223

Age, Sex Impetuous Factor for Thyroid Dysfunction, Oxidative Stress among Urban and Rural Population of Central India

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The total burden of thyroid disorders in India is 42 million. Central Indian population showing obvious incidences of thyroid dysfunction. With the perception of age as a factor, study has been carried out under two groups Group I: Below 35 years of age, Group II: Above 35 years of age, at tertiary care centre. 60% hypothyroid patients were of urban, while 40% belong to rural population. Of 100 newly diagnosed hypothyroid patients, 55 patients among total were above 35 years of age & 45 below 35 years of age. Biochemical parameters, lipid profile, TAC have been estimated for each group in a prospective mode i.e., before initiation and after six weeks of therapy. Result showed higher incidence amongst urban population, females being more susceptible with advancement of age. Dyslipidemia reported in Group II (TC 232.84 $\pm$ 47.01 $P < 0.001$, LDL137.6 $\pm$ 32.01 $P < 0.001$), Oxidative stress was found in both groups I & II (TAC1.591 $\pm$ 0.497, 1.588 $\pm$ 0.500). Screening of Hypothyroidism is very essential after 35 years of age, and apart from thyroid harmonization, antioxidative supplement should be taken account of, to avoid cardiac problems & oxidative stress.

P224

Air Transport Fuel Exposure amongst Ground Personnel in Aviation Sector

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Approximately 60 billion gallons of kerosene-based jet aircraft fuel or Air Transport Fuel (ATF/JP-8/Jet A1) is annually consumed worldwide. ATF has been recognized as a major source of hazardous chemical exposure for refuelling and air craft maintenance workers world over. In India, thousands of military and civilian ground personnel are occupationally exposed to this ATF. Exposure to ATF may occur due to raw fuel, vapour phase, aerosol phase, or fuel combustion exhaust. A number of studies have reported acute or persisting health effects from acute, sub-chronic or chronic exposure to constituent chemicals of ATF or its combustion products. Studies of such exposures in animal models have found major adverse effects on various organ systems including carcinogenic effects. Based on these studies, international agencies have defined Permissible Exposure limits (PEL) of total hydrocarbon vapours in the breathing zone air of aircraft maintenance staff. In India, there is no study conducted yet to evaluate the fuel exposures in personnel handing aviation fuels and efficacy of protective measures used in both civil and military aviation. There is also a complete absence of defined safe exposure standards for monitoring safety of populations exposed to these fuels. In this review the potential health hazards of exposure to ATF, various international studies and the PELs defined by them will be discussed in detail.

P225

Erythrocyte L-Cysteine Influx in Subjects of COPD

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COPD is considered to be one of the most distressful conditions in Respiratory Medicine. Emphysema, chronic bronchitis, chronic bronchial asthma is the common cause of COPD. Worldwide, COPD affects 329 million people or nearly 5% of the population. In 2012, the disease ranked as 3rd leading cause of death, killing over 3 million people. Cigarette smoke and its condensates enhance oxidant induced injury to A 549 human type II alveolar epithelial cells, which was reversed by adding GSH extracellularly and worsened by depleting GSH intracellularly with buthionine sulfoxamine. L-cysteine plays a vital role in cellular Glutathione metabolism. The aim of our study was to measure the L-cysteine influx in erythrocyte of the

affected patients and controls to assess the redox status in erythrocyte. 24 patients were selected from the Department of Medicine (TB & Chest Unit), MGM Medical College & Hospital, Kishanganj, Bihar. 24 controls were also selected from the population not suffering from any vital disease. 3 ml blood was collected in EDTA vial to essay the L-cysteine influx. We observed a decreased uptake of L-cysteine in the erythrocyte of patients of COPD as compared to the control. Diminished uptake of L-cysteine by erythrocyte indicates altered redox status which may be due to less synthesis of Glutathione in erythrocyte.

P226

Activity of Lecithin Cholesterol Acyl Transferase and Apolipoprotein A-I in Newly Diagnosed HIV Patients

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India has the third highest number of HIV patients in the world. HIV is the fourth leading cause of death worldwide. Metabolic complications continue to play a major role in the management of HIV infection. The potential clinical and pathological consequences of HIV associated hyperlipidemia are not completely known, but several studies reported an increased risk of coronary artery disease in HIV positive individuals. The aim of the study was to study the levels of Apo A-I and LCAT activity in newly diagnosed HIV patients. Present study includes 150 newly diagnosed HIV patients from Integrated Counseling and Testing Centre (ICTC), BIMS Hospital, Belagavi and 150 age and sex matched healthy controls. Fasting venous blood samples were collected. Serum was used for estimation of LCAT activity, Apo A-I and HDL. LCAT activity was assessed by measuring the difference between esterified and free cholesterol by digitonin precipitation method. Apo A-I was measured by immunoturbidemetric method. The LCAT activity and Apo A-I levels were significantly ($p < 0.05$) decreased in newly diagnosed HIV patients when compared with healthy participants. The reduced LCAT activity, Apo A-I and HDL may be associated with a reduction in reverse cholesterol transport and this may be a risk for the development of atherosclerosis in HIV.

P227

Evaluation of Lipid Profile and Lipid Peroxidatio (MDA) in Chronic Smokers

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Epidemiological studies show that smoking is one of the leading causes of human mortality and morbidity. Various toxic chemicals and free radicals of cigarette smoke increase oxidative stress by peroxidation of membrane lipids and other cellular components, leading to cardiovascular diseases, cancer and COPD. The aim of the study was to assess the effect of smoking on lipid peroxidation, Malondialdehyde (MDA) level and serum lipid profile. A total of 50 healthy male smokers and age matched 50 healthy controls were recruited for the study. Smokers were categorized according to number of pack years, varied from 10 to 14 years (mild), 15 to 19 years (moderate) and $\geq$ 20 years (heavy). TBARS method was used to measure serum MDA levels while fasting lipid profile was estimated using standard methods. Mean levels of total cholesterol, LDL-C and MDA were significantly increased ($p < 0.05$) among all the groups of smokers compared to the controls. No significant change was observed in Triglycerides, VLDL and HDL levels in any individual group of smokers compared to the controls ($p > 0.05$). Group of mild, moderate and heavy smokers showed significantly increased mean level of MDA when compared to each other. Dyslipidemia and increased lipid peroxidation observed in smokers are powerful contributing factor for premature development of cardiovascular and other diseases.

P228

Renoprotective Effects, Protein Thiols and Evaluation of Liver Glycogen of Different Fractions of Heart Wood of *Pterocarpus Marsupium* on Alloxan Induced Diabetic Rats

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Diabetes mellitus is a worldwide illness, which has been constantly affecting the human race, irrespective of the socioeconomic profile and geographic location. Oxidative stress is known to be a marker of molecular and cellular tissue damage mechanism in a wide spectrum of human diseases. In the present study, we aimed to evaluate the effects of different fractions of heart wood of *Pterocarpus marsupium* on antioxidant enzyme such as protein thiols and also check the efficacy of the extract for the protection of the renal function by assaying urea, uric acid and creatinine in alloxan induced diabetic rats. This study also investigated the levels of liver glycogen which is considered as the best marker for assaying the hypoglycemic activity of any drug. Diabetes was induced by administering alloxan which was dissolved in saline, while the normal control group was given the vehicle (propylene glycol). Estimation of urea, uric acid and creatinine along with protein thiols was done on day 30 only. Treatment of 30 days with various extracts (75 mg / kg body wt) significantly lowered the levels of protein thiols which probably represent increased utilization for neutralizing free radicals. There was no significant increase in the levels of renal parameters in experimental treated groups which revealed that the employed dose of the extract is non toxic to kidney. There was also a significant decrease in the glycogen content in insulin and alcohol treated groups and should be encouraged in the treatment of diabetes mellitus.

P229

Association of HLA-DRB1Alleles and Auto antibodies in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic autoimmune disorder of unknown aetiology resulting in inflammation of the synovium, cartilage and bone. The genetic contribution to RA pathogenesis has been predicted to be $\sim 60\%$ and the human leukocyte antigen (HLA) has consistently shown the strongest genetic association with RA. Autoimmunity in RA is represented by the presence of rheumatoid factor (RF) and anti-cyclic citrullinated peptide (Anti-CCP). The main objectives of this study were to study the distribution of *HLA-DRB1* allele frequencies in RA patients and controls and to analyze the association between *HLA-DRB1*shared epitope (SE) alleles and autoantibodies in RA as susceptible markers. The study design consist of two groups, control (healthy individuals, $n = 20$) and test (RA patients, $n = 20$) following the proposed inclusion and exclusion criteria. The genotyping of *HLA-DRB1* was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) method. The autoantibodies in serum (RF & Anti-CCP) were determined by *invitro* quantitative ELISA method. Chi-squared and Students's *t*-test were used in the statistical analysis. Differences were considered to be significant at $P < 0.05$. In our RA patients *HLA-DRB1**04,*10,*14 allele frequencies were higher than controls. In contrast, *DRB1**11 were more frequent in control population indicating a possible protective effect. There was no statistically significant difference observed in *DRB1**1, *03, *07, *08, *13 & *15 between patients and control groups. The frequency of SE positive alleles were higher in RA patients (62.5%), compared to control group (12.5%). The frequencies of anti-CCP and RF were higher in SE-positive patients when compared to SE-negative patients. Our results indicated that the *HLA-DRB1**04,*10,*14 alleles, were significantly associated with RA susceptibility. Our findings also support the protective role of*DRB1**11 alleles in the pathogenesis of Anti-CCP positive RA.

P230

Comparison of Lipid Profile and Thyroid Hormone Level between Pre and Post Menopausal Hypertensive Women

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Hormonal changes of menopause may play a role in high Blood Pressure. Androgen and Sex hormone finding, globulin (SHBG) levels have been associated with risk of cardiovascular disease in Pre and Postmenopausal women An increase in Circulating androgens appears to be associated with insulin resistance and predictor of diabetes mellitus. In the present study we compared the serum lipid profile and thyroid hormone level between 50 premenopausal and 50 post menopausal hypertensive women. All parameters were measured by standard methods. It was found that the lipid profile and TSH level were elevated in post menopausal hypertensive women as compare to premenopausal hypertensive women. Serum cholesterol, triglyceride, VLDL and LDL-C levels were significantly ($P < 0.0001$) high while HDL-C level were significantly ($P < 0.0001$) lower in post-menopausal compare to premenopausal hypertensive women. TSH level was significantly ($P < 0.0001$) increased while T3 & T4 hormone level were significantly ($P < 0.0001$) decrease in postmenopausal compare to premenopausal hypertensive women. It is concluded that elevated LDL and the reduction of cardio protective HDL and thyroid hormone level T3 and T4 indicate that menopause is an independent risk factor for developing cardiovascular disease.

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Serum Free Fatty Acid Levels in Neonatal Sepsis

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Sepsis is the commonest cause of neonatal morbidity and mortality; responsible for 30 – 50 % of total neonatal deaths in developing countries. It is considered a life – threatening clinical emergency that necessitates urgent diagnosis and treatment. There are controversial reports on fatty acid metabolism in various animals and human during sepsis. The aim of this study was to compare the serum free fatty acid (FFA) levels in neonates with and without sepsis. The FFA levels increased considerably during sepsis ($P = 0.0043$) as did the total leucocyte count (TLC) ($P = 0.017$). 8 out of 20 controls and 36 out of 40 cases of neonatal sepsis had high c – reactive protein (hs-CRP). The correlation between FFA versus TLC was negative in cases ($r^2 = 0.004$) and positive in controls ($r^2 = 0.366$). During sepsis there is an increase in plasma catecholamine concentration which stimulates adipose tissue FFA release. This results in enhanced uptake of FFA by liver. Endotoxemia causes release of other cytokines like Tumor necrosis factor (TNF) and Interleukin – 1 (IL -1) which suppress lipoprotein lipase (LPL) synthesis, VLDL – receptor and carnitine palmitoyl Transferase I (CPT -1) activity. All these combined with altered glucose metabolism by organs like heart, kidney, skeletal muscle result in alterations in the lipid and carbohydrate metabolism. Our study showed an increase in serum FFA levels with sepsis and an inverse relation with TLC. This can be used as a diagnostic / prognostic marker and nutrition strategies developed based and this to prevent metabolic complications and organ failure during sepsis.

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Incompatibility of Cow's Milk Proteins (Animal Proteins) with Human Proteins

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When an infant is weaned from mother's milk, it has traditionally been the practice to replace the mother's milk with cow's milk. This can have unpleasant consequences. Recent research

has shown that cows milk protein often persists and it is misdiagnosed by many clinicians. CMA is a global challenge. People who argue against milk consumption do so for a number of reasons. Let us take a look at some of the main ones: Milk allergy reasons, Lactose intolerance and compatibility of cow's milk with the human body. The aim of this study was to evaluate the clinical and immunological characteristics of a group of infants with persistent CMPI. Whether or not your patients reacts adversely to cow's milk may depend on several factors. Milk may be a healthy, healing food for you, but a seriously damaging food for your next door neighbor. Expected to have milk allergies were followed from 2 years of age to 15 years of age The study was done at children attending the Out Patient Department of ENT AND ALLERGY CENTRE (INDIA) Panchkula. Children with off and on complains of angioedema, wheezing, rhinitis, dry cough, vomiting, laryngeal edema, acute asthma with severe respiratory distress were taken for study. Late reactions due to cow's milk allergy are atopic dermatitis, chronic diarrhea, blood in the stools, iron deficiency anemia, gastro esophageal reflux disease, constipation, chronic vomiting, colic, poor growth (food refusal). In our trials majority of the patients showed sensitivity to CMA who had respiratory allergies(30.2%) cutaneous sensitivity(50.5%)and gastrointestinal manifestation(20.3%.)Many Children continue to have chronic symptoms even the original problem may disappear. That means some children may have an allergic tendency that persists in later age. The patients underwent specific IgE levels in blood for milk. and underwent clinical evaluations. As controls we followed 50 infants. Clinical presentation changed over time. At onset symptoms were prevalently gastrointestinal, while at the end of the study there was an increased frequency of wheezing and constipation. Persistent CMPI forms are characterized by family history of allergies. The symptoms and target organ changes from time to time. There are chances of multiple food intolerance in allergic patients.

P233

Maternal Serum and Cord Blood Leptin Levels in Intrauterine Growth Restricted and Appropriate for Gestational Age Pregnancy

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Leptin, a 16 kDa protein hormone is known to be involved in the regulation of adipose tissue mass of the body. Recent studies have indicated that leptin is a gestational hormone with a possible role in regulation of fetal growth. This study was done to find out whether maternal and cord blood leptin correlate with fetal outcome. This cross-sectional study was carried out in 100 pregnant women of age 22–40 years with singleton pregnancy. The study group included fifty Intra-uterine growth retarded pregnant (SGA) women and control group included fifty women with AGA pregnancy. Maternal and cord blood were collected during delivery from all subjects for estimation of leptin. Clinical evaluation included birth weight, and APGAR score at 1 minute and 5 minutes. Cord blood leptin levels in Intrauterine growth retarded pregnancies were found to be significantly lower (9.16 ± 8.37 ng/ml) as compared to normal pregnancy (17.90 ± 16.66 ng/ml) $p = 0.001$). Maternal serum leptin levels were not found to be significantly different in the two groups (42.54 ± 29.41 ng/ml in normal pregnancy vs 40.26 ± 31.59 ng/ml in cases ($p = 0.709$). Both maternal and cord blood leptin levels showed a positive correlation with birth weight but it was found significant only in IUGR cases ($p = 0.031$). The association between cord blood leptin and birth weight suggests a pivotal role of fetal leptin in regulating fetal growth and development.

P234

Study of Transcobalamin II Gene Polymorphism in Stroke Patients in Indian Population

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Stroke is a complex multifactorial disease influenced by several genetic and environmental factors. Vitamin B12 deficiency is a major public health problem in Indian population due to adherence to strict vegetarian diet. Transcobalamin II is a key protein involved in intracellular transport of vitamin B12. So, genetic variation in transcobalamin II gene may lower concentration of intracellular vitamin B12 and may increase the susceptibility of stroke. The objective of the present study was to explore the effect of transcobalamin II gene polymorphism upon susceptibility to strokein Indian population. A case-control study was conducted in Department of Biochemistryand Medicine, VMMC and SJH, New Delhi, which included 32diagnosed cases of stroke and 32 age and sex matched healthy control subjects. Genomic DNA isolation was done by DNA extraction Kit. The genotyping fortranscobalamin II SNP (rs4820889 (A→G)) was done by Allele Specific Polymerase chain reaction. Among cases, genotype frequency of AA is 75%, AG is 18.8%, GG is 6.3% and allelic frequency of A is 84.4% and G is 15.6%. Among controls, genotype frequency of AA is 93.8%, AG and GG is 3.1% and allelic frequency of A is 95.3% and G is 4.7%. Pearson Chi-Square p-value = 0.0389, OR = 5.0;95% = 0.96–25.78. In our study we have found that transcobalamin II gene SNP rs4820889is significantly (p-value <0.05) associated with the risk of stroke.

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Study of Serum Ferritin in Smokers and Non-smokers

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Smoking is one of the biggest public health problems throughout the world. The most common method of smoking is through cigarettes. Smoking leads to diseases affecting the heart and lungs and major risk factor for heart attacks, chronic obstructive lung disease, and emphysema and cancer. Serum ferritin was observed to be one of the strongest indicators of the presence and progression of carotid artery disease. The present study was aimed to understand the influence of smoking in lipid profile and Ferritin in chronic cigratte smokers. The stusy was aimed to study serum ferritin levels in smokers and non smokers. The study group included 100 smokers age between 20 and 35 years and compared with 100 age and sex matched non-smokers were recruited from MHC at SRMMCH & RC. Blood samples were analysed for the following parameters Serum Ferritin,

Serum Iron, TIBC and Fasting lipid profile: total cholesterol, Triglycerides, HDL–C, and LDL-C. All the statiscal analysis was performed using statistical package SPSS. Serum Ferritin levels in smokers is observed to be slightly increased in smokers when compared to controls. A significant increase in serum cholesterol, Triglycerides, VLDL cholesterol is observed with HDL-C level showed significant decrease compared to Non-smokers. An increase in Serum ferritin in smokers may be a strong independent risk factor for premature coronary heart disease.

P236

Effect of Yoga on Biochemical Profile Among Arthritis Patients

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Through understanding this study by the way of Laboratory Investigations with effect of yoga, the data and result outcome of the study will play an immense role in health promotion and prevention of Arthritis disease. This is a hospital bases study considering a small group of population in Jaunpur city, Uttar Pradesh, India. Objective of present work is to find out the effect ofyoga on biochemical profile among arthritis patients. This study, consisted of 50 older age (34 male and 16 female) in the age group of 45 – 60 years, was carried out to a biochemical parameter under Arthritis is done at Seven different time {before yoga, duration of yoga (after 2 months, 4 months, 6 months, 8 months, 10 months, 12 months} during the period of April 2014 –April 2015. Including age and sex, the biochemical parameter under arthritis panel which are used in this study are Serum Uric Acid, Blood Urea, SGOT, SGPT, ALP, CRP, RA Factor, ASO Titer. The major findings are indicating the yoga effects on biochemical parameter having highly associated with their age. The main aim of this paper to investigates the effect of yoga on biochemical profile among arthritis patients. This study, originally covered 72 older persons (47 males and 25 females) consisted in the age group of 45 – 60 years and was carried out to a biochemical parameter under arthritis is done at seven different time {before yoga as base line, duration of yoga(after 2 months,4 months,6 months, 8 months, 10 months & 12 months} during the period of April-2014 to April-2015, at the SSRM Paramedical Institute, Jaunpur district, Uttar Pradesh, India. Among all the 72 persons, only 34 males and 16 females were continued the full six follow up. The biochemical parameters underarthritis panel that are used in this study are Serum Uric Acid (for male 2.5–7.0 mg/dL & for female 1.5–6.0 mg/dL), Blood Urea (20–40 mg/dL), SGOT(8–40 u/L), SGPT (5–35 u/L), Alkaline phosphatase(ALP; 60 to 120 u/L), C-Reactive Protein (CRP) 0 to 6 5 mg/L, Rheumatoid-Factor (RA $<$ 20 IU/ml) & Anti-streptolysin O(ASO-Titre 0 to 200 mg/dL). Data was analyzed with relevant descriptive statistics like, frequency, mean and percentages were calculated for presentation of data. Paired t test was used to compare the continuous variables from baseline to follow-up. Mann-Whitney U test, a nonparametric test, was used to compare the differences in various parameters before and after intervention between the two groups. Amongst 50 patients with arthritis symptoms, 34 (68%) were males and 16 (32) were females, making the ratio nearly 2:1 (M: F). Mean age of onset was 48.60 $\pm$ 4.64 years. From Table 1, the value of all biochemical profile is continuously decreases with the practices of yoga. Similarly from the Table 2, at the baseline period only 22 (44%) persons having all arthritis profile test in normal range, after the 12 months yoga practices this value increases up to 42 (84%) this shows that intervention of yoga having a positive effect to control arthritis. From the Table 3, this shows that only 35 (70%) persons are find in normal range for serum uric acid test at baseline period and 49 (98%) after the significantly intervention of one year yoga practices (Cochran's Q value = 44.14 & P-value = 0.001). Blood urea, SGOT, SGPT & ASO-Titre profile tests are showing positive response but this are not statistically significant. ALP (Cochran's Q value = 23.60 & P-value = 0.001), CRP (Cochran's Q value = 42.82 & P-value = 0.001) and RA Factor (Cochran's Q value = 41.12 & P-value = 0.001) profile tests shows that yoga treatment is effective. At last the research report and the paper consisting upon the different biochemical tests at different levels, ages and gaps of time says that the arthritis can be reduced even can be cured through yoga, especially in the age group between 40–65 where the chances of curability is very less through medication, but the actions of yoga must be done in proper guidance of experts.

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Polymorphic Study of Inflammatory Cytokine Genes in North Indian Rheumatoid Arthritis Patients

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Rheumatoid arthritis (RA), a common autoimmune disease, is a destructive arthropathy. The etiology of RA remains unknown and it is proposed to be a multifactorial disease. RA is associated with altered expression of pro- and anti-inflammatory cytokines. The aim of the study was to investigate the association between promoter polymorphisms of TNF-α, IL-1B and IL-10 genes with susceptibility of disease in North Indian patients with RA. Fifty RA patients with age and sex matched control were recruited and genotyped. Single nucleotide polymorphisms of TNF-α (-308), IL-1 B (-511) and IL-10 (-1082) were studied in RA patients and healthy controls by RFLP method. The biochemical parameters were determined by standard laboratory methods using commercially available kits. Hemoglobin was significantly lower in RA patients while ESR and level of biochemical parameters specific to RA (RF, anti-CCP and CRP) and serum levels of TNFα, IL-1β and IL-10 were significantly higher in RA patients as compared to healthy controls. Dyslipidemia was observed in RA patients. In TNFα -308 the frequency of mutant allele (A) was higher in RA patients in comparison to the healthy controls. In case of IL-1B (-511) the frequency of mutant allele (T) was higher in patients with RA than those in control group and in IL-10 (-1082) gene the frequency of mutant allele (G) was higher in RA patients in comparison to the healthy control. Single nucleotide polymorphisms at positions -308 of TNF-α gene, -511 of IL-1B gene and -1082 of IL-10 gene may be associated with disease susceptibility.

P238

Haemoglobinopathies in Jammu Division

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Haemoglobinopathies is a set of disorders having abnormal globin protein. Disorders range from thalasemia to many haemoglobin variants with no, mild or severe consequences for the carrier and the sufferer. The aim of the study was to study the prevalence of haemoglobinopathies in Jammu Division of Jammu and Kashmir State. The present laboratory-based retrospective study was conducted for a period of two years from January 1, 2013 to December 31, 2013. Data of 543 patients, who had come to the laboratory for their haemoglobin electrophoresis, was compiled and studied. Complete blood count and Haemoglobin electrophoresis were done for all the patients. CBC was carried out on HMX (Beckman Coulter) and Hb electrophoresis was done on D10 (BIO RAD) (HPLC). Out of 543 patients, 175 (32.23%) had abnormal haemoglobin pattern. Spectrum of haemoglobinopathies prevalent in descending order were 13.99% β-thalassemic trait, 6.26% α-thalassemic trait, 4.6% elevated fetal haemoglobin, 2.57% false elevation of haemoglobin A2 because of Mean Corpuscular Volume (MCV), 1.29% β-thalasemic major, 0.93% haemoglobin S homozygous, 0.74% borderline haemoglobin A2, 0.37% each had haemoglobin E trait and fetal haemoglobin with β-thalassemia or thalassemia major and 0.18% each had reduced haemoglobin A2, intermedia of α-/β-thalassemia, haemoglobin S trait and haemoglobin S/haemoglobin D double heterozygous. High prevalence ofhaemoglobinopathies in Jammu divisionmade the disease a major public health problem in our population. Population screening, genetic counseling and prenatal diagnosis can prevent these genetic disorders.

P239

Study of Serum Adenosine Deaminase Activity in HIV Patients with Antiretroviral Therapy

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India has the third highest number of estimated people living with HIV in the world. HIV infection is characterized by replication by aberrant immune activation and persistent inflammation. Recent studies show a causal relationship between adenosine deaminase (ADA) activity and normal immune function. ADA has a cytokine-like costimulatory role in T cell proliferation, which is independent of catalytic activity. The objective of the study was to estimate adenosine deaminase activity (ADA) in HIV patients before and after 3 months interval of antiretroviral therapy (ART) up to 9 months. The study was included 150 HIV positive patients between age group of 20–50 years from ICTC (Integrated Counseling and Testing Centre) and ART centre of Belagavi Institute of Medical Sciences, Hospital, Belagavi. Venous blood samples were collected in plain bulb to estimate serum ADA activity, before and after 3 months interval of ART. Serum ADA activity was estimated using colorimetric method of giusti and galanti. The study showed activity of serum ADA was significantly raised before ART and after 3 months interval of ART there was no change in CD4 cell count. After 6 months of ART increased ADA activity started declining and comes near to normal after 9 months of ART. Tha study concluded that Elevated serum adenosine deaminase activity in HIV patients is an indicator of T-cell activation. With antiretroviral therapy there is reduction in viral load and T-cell activation, as a result there may be gradual decrease in ADA activity. Hence serum ADA activity along with other markers, can be used as a diagnostic and a prognostic marker to monitor response to antiretroviral therapy in HIV patients.

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Correlation of Arterial Blood Gas Analysis in Medicine Intensive Care Unit Patients to Mortality

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It is difficult to predict outcome of most of critically ill patients admitted daily in Medicine Intensive Care unit (MICU). Management of most serious and acute diseases involves variable degree of acid-base disorder which needs to be identified and treated rapidly. Early and accurate identification of patients requiring aggressive medical intervention is essential to have improved outcome for survival. The objective of the study was to correlate initial blood gas analysis in MICU patients to their outcome. 300 patients admitted to the MICU of DMC & H, Ludhiana irrespective of their underlying diagnosis were included. They were then divided into survivors and non survivors depending upon mortality within 7 days of admission to MICU. Heparinized samples were analyzed on Blood Gas Analyzer ABL 800 (RADIOMETER). In the study pH, pCO_2, SO_2, glucose, lactate, bicarbonate and chloride showed statistical difference between survivors and non-survivors. Comparison of survivors and non-survivors for age, pH, pCO_2, SO_2 glucose, lactate, bicarbonate and chloride showed relevant differences. When dividing the group of patients on the basis of reference range, acidosis, hypercapnia, hyperoxia, hyperkalemia, hyperglycemia, hyperlactataemia, low bicarbonate and hypochloremia were all related to high mortality in comparison to normal levels in survivors. The study concluded that arterial blood gas analysis in patients presenting to the intensive care unit can help in predicting adverse outcome. Aggressive medical intervention can help in survival of atleast some of these patients.

P241

Therapeutic Drug Monitoring of Rifampicin and Isoniazid: An Indian Perspective

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Rifampicin and Isoniazid are the two most crucial drugs used for treatment of drug susceptible tuberculosis (TB). Low or

abnormal plasma concentrations of anti-tuberculosis drugs can be a major reason for treatment failure or emergence of drug resistance. The objective of the study is to perform therapeutic drug monitoring (TDM) of rifampicin and isoniazid amongst Indian patients on first line therapy for tuberculosis. In-house standardized high performance liquid chromatography (HPLC) methods were used for estimation of plasma rifampicin and isoniazid levels. Peak levels i.e 2 hour post dose ingestion were collected from 25 patients who were on first line therapy for atleast 7 days. Patients were regarded as clinically improved or partial responders on basis on a detailed clinical follow-up after 2 or more months by observing all the TB symptoms. The study showed a median plasma level of 10 mg/l for rifampicin and 4.84 mg/l for isoniazid were observed in the study group. About, 40% and 28% had sub-therapeutic levels of rifampicin and isoniazid respectively; 32% had toxic isoniazid levels while no patient was in the toxic range for rifampicin. Only 15 patients (60%) improved clinically while 10 patients (40%) were partial responders to the therapy. Nine patients were partial responders despite having either one of the drugs in the therapeutic or toxic range. The preliminary results of this ongoing study suggest abnormal concentrations of rifampicin and isoniazid are common and a major concern. TDM may help identify patients at risk of delayed response and optimize the drug doses to obtain adequate clinical response.

P242

Association of Serum Total Cholesterol with Sensorineural Hearing Loss – A Hospital Based Study

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Deafness is a major social and educational handicap and is the most prevalent sensory impairment worldwide. The aetiology and pathogenesis of sensorineural hearing loss (SNHL), a type of deafness are not fully understood. The objective of the study was To find out the association between total cholesterol and sensorineural hearing loss. This hospital based case control study included a total of 108 subjects (54-control, 54-clinically diagnosed cases of SNHL), aged 18 years and above. Total serum cholesterol was estimated by Cholesterol SR kit (CHOD/PAP method) using semi auto analyser MICROLAB 300 (MERCK). Statistical analysis included Independent samples T test and ANOVA. In the study increased serum total cholesterol was significantly associated with sensorineural hearing loss ($p < 0.05$). The study suggests that a high serum total cholesterol level may play a role in the pathogenesis of sensorineural hearing loss.

P243

Study of Antioxidant Level and Serum Creatinine in Hypertensive Disorder of Pregnancy

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Hypertensive disorders of pregnancy is a common complication of late pregnancy is a major cause of renal damage during pregnancy. It includes: Pre-eclampsia and Eclampsia. Pre eclampsia general prevalence is between 2 and 3% of pregnancy. It is a common cause of both maternal and perinatal morbidity & mortality in both developed and developing countries. Eclampsia is an acute and life-threatening complication of pregnancy. It includes seizures and coma that happen during pregnancy but are not due to preexisting or organic brain disorders. The aim of this study was the assessment of serum creatinine and serum uric acid level in hypertensive disorders of pregnancy (pre-eclampsia & eclampsia). The present study includes 50 patients of preeclampsia and eclampsia and 50 healthy controls. Blood sample were taking and following investigation were done estimation of serum creatinine by Jaffe reaction without deproteinisation, kinetic method and estimation of serum uric acid by Uricase/Trinder method. This study showed the significant increased in serum uric acid and, significant increased in creatinine cases as compare to healthy controls. The concluded that increased Serum uric acid and serum creatinine level in associated with in hypertensive disorders of pregnancy (pre-eclampsia and eclampsia).

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Comparison of Proliferating Capacity and Morphology of Mesenchymal Stem Cells Isolated from Human Umbilical Cord Wharton's Jelly and Cord Blood

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Mesenchymal stem cells are emerging to be a popular subtype of adult stem cells. Human umbilical cord Wharton's Jelly (hUCWJ) and Human cord blood (hCB) are rich and promising sources of MSCs due to its close association with embryonic tissue. It is a relatively non-controversial, easily available source of human tissue. MSCs derived from these sources are multipotent and have the ability to differentiate into ecto-dermal, meso-dermal and endodermal lineage and is emerging as a promising tool for regenerative medicine. The aim of the study was to compare the early characteristics of MSCs isolated from hUCWJ and hCB. The MSCs from hUCWJ and hCB were isolated by explant culture and ficoll density gradient respectively. The complete medium used were Dulbecco's modified Eagle's medium F12 (DMEM F12) and fetal bovine serum (FBS) 10% for hUCWJ and Iscove's modified Dulbecco's medium (IMDM) and FBS 10% for hCB. Cells were sub-cultured when 70–80% confluence was seen. They were then compared for morphology, proliferation rate and colony forming units (CFU). In culture, MScs from hCB started to appear spindle shaped within 5–7 days and hUCWJ cells started migrating within 7–9 day from the explants. The MSCs derived from both the sources grew long, spindle shaped cells with prominent nuclei and also formed colonies on subcultures in plastic-ware, but proliferation rates and CFU were higher for cells isolated from hUCWJ when compared with hCB. The study concluded that MSCs isolated from hUCWJ have greater Colony Forming and proliferating capacity than hCB.

P245

Prolactin in Lichen Planus: A Case-Control Study

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Lichen planus (LP) is a common chronic inflammatory disease involving the skin and mucous membranes. Its etiology is thought to be autoimmune. Various cytokines (such as IL-2, IL-4, IL-6, IL-10, TNF-α, interferon (IFN)-α, IFN-γ and transforming growth factor-β1) are involved in LP similar to psoriasis. Prolactin, a peptide hormone secreted from pituitary, has a vital role in autoimmune related diseases like lupus erythematosus, rheumatoid artharitis, and psoriasis. Prolactin may be good biomarker of severity of disease with similar autoimmune inflammatory etiopathogenesis. So we conducted the current study to evaluate serum levels of prolactin in cases of lichen planus to evaluate any correlation of prolactin with lichen planus. We conducted a prospective case control study from December 2013 to march 2015on 25 patients of lichen planus and 25 age and sex match healthy control subjects. We measured serum prolactin level by ELISA technique. The study showed total 50 subjects (25 cases and 25 controls) were evaluated in this study. Demographic parameters (such as age, sex, body mass index) were comparable between the groups. Serum prolactin levels was significantly higher in compared with the control group (p value <0.05). The mean $\pm$ SD of the serum prolactin were 18.9 ± 6.8 ng/ml and 11.4 ± 6.0 ng/ml in patients with Lichen planus and control respectively. The study concluded that prolactin may play a role in the pathogenesis of lichen planus and may serve as a biological marker of disease activity in patients with lichen planus. Further large scale studies are needed to prove our findings.

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Study on the First and Second Trimester Maternal Serum Magnesium and Nitric Oxide Levels for the Early Detection of Preeclampsia

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Preeclampsia is a pregnancy specific syndrome of elevated blood pressure and presence of protein in urine after 20 weeks of gestation in a woman who was previously normotensive. Even though, the clinical manifestations occur after 20 weeks of gestation, the underlying cause exists from the time of implantation of embryo. Maternal endothelial dysfunction is a characteristic of the disease. Studies have revealed that serum nitric oxide levels are decreased in women having preeclampsia. Changes in extracellular magnesium content are able to modify the production and release of nitric oxide (NO). The aim of the study was to evaluate serum magnesium and nitric oxide levels in the first and second trimesters and to identify if this could serve as an early marker for the detection of preeclampsia. Pregnant women attending the antenatal clinic of Dr. TMA Pai hospital, Udupi, from the first trimester and willing to participate in the study were included. First and second trimester blood samples were collected and they were followed up till delivery to check if they developed preeclampsia. Selection of cases ($n = 14$) and controls ($n = 99$) were made based on the ACOG guidelines. Serum magnesium was estimated by Calmagite kit method and serum nitric oxide by Griess method. In the study nitric oxide levels in both trimesters were lower in the case group. From the ROC curve, it was observed that women having first trimester nitric oxide levels $\leq$12.6 μmol/L are two times more likely to develop preeclampsia. The study concludes that serum nitric oxide may indicate the development of preeclampsia after 20 weeks.

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A Comprehensive Study of Serum Cacium, Vitamin D and Parathyroid Hormone with Respect to Occupational Lead Exposure in Jewellery Workers in Dhaka, Bangladesh

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Jewellery utilizes lead either directly or as a base metal. Costume jewellery requires lead before molding and plating the product with valuable metals. Therefore, such ornaments have a great potential to release heavy metals having health hazards. Also, jewellery makers engaged in preparing German silver, an alloy, apply lead in smelting, alloying, rolling and milling silver wires and pieces. The metal is taken up by blood, soft tissues and bone. The biological effects of lead are dependent upon the level and duration of exposure. Lead inhibits three enzymes of heme biosynthesis- δ-amino-levulinic-acid dehydratase (ALAD), coproporphyrin oxidase, and ferrochelatase, impairing heme synthesis and depressing serum level of erythropoietin. Lead also decreases erythrocyte survival through inhibition of membrane bound Na + K + ATP-ase, resulting in decreased hemoglobin synthesis. Lead exposure also affects calcium metabolism and impair the synthesis of Calcitriol. In the present study, jewellery makers from Dhaka, Bangladesh, were shown to have significantly high levels of lead, protein, albumin, and parathyroid homones in their blood, and significantly high amount of zinc-protoporphyrin and δ –aminolevulinic-acid in their urine. The control group, on the other hand showed significantly higher amounts of calcium (both total and ionized form) Vitamin D3 and non-activated erythrocyte ALAD in their blood, along with hemoglobin. It might be due to inhibition of 1-α-hydroxylase enzyme in renal tubules. Lead causes nephro-toxicity and inhibits 1-α- hydroxylase enzyme leading to decreased calcitriol synthesis resulting in impaired calcium absorption across gastro-intestinal tract and renal tubules. Low Vitamin D3 and significantly increased Parathyroid hormone (PTH) in study group has been found.

P248

Efficacy of Estimating Pleural Fluid Cholesterol in Diagnosing Tubercular Pleural Effusion

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Tuberculous pleural effusion (TPE) is diagnosed by biopsy or PCR on clinical suspects and delay in this is still frequent in India. ADA is not readily available mostly in hospitals with limited laboratory facilities. Pleural fluid cholesterol has been used to classify exudates and transudates as it misclassifies fewer cases than any other Light's parameters. The objective of the study was to evaluate the utility of cholesterol in lymphocytic exudates in diagnosing TPE in a region of high prevalence of PTB which has never been done before. The study was carried out on 80 patients with PE. Fluid classified as lymphocytic exudates based on light's criteria and lymphoytic proportion >0.75 were differentiated into tubercular and non-tubercular PE based on biopsy or PCR. Fluid ADA and fluid cholesterol were done in both the groups. In the study 49 were positive for TPE. Fluid ADA and fluid cholesterol levels were significantly different in tubercular and non-tubercular PE cases. Fluid cholesterol correlated positively with fluid ADA. Sensitivity, specificity, PPV, NPV with fluid cholesterol value of 50 mg/dL as cut off were 95.9 %, 100 %, 1005, 84.6 % which was better than using fluid cholesterol value of 60 mg/dL as cut off and fluid ADA. The study concluded that fluid cholesterol estimation could be a feasible option for cheaper diagnosis of TPE and it correlates with fluid ADA. The good accuracy of this method makes it a promising diagnostic tool that could be used for diagnosis of TPE in area where disease has high prevalence. A negative result excludes TPE with a high degree of certainty.

P249

Impaired Angiogenesis and Pregnancy Outcome in Patients with Hypertensive Disorders of Pregnancy: A Pilot Study in an Indian Population

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The objective of the study was to assess the angiogenic and antiangiogenic factors in various types of hypertensive disorders of pregnancy and to correlate with the pregnancy outcome. Plasma levels of soluble Vascular Endothelial Growth Factor Receptor 1 (sVEGFR1), soluble Endoglin (sENG), Transforming Growth factor beta (TGF-b), VEGF, Placental Growth Factor (PLGF), Matrix metallo proteinases-9 (MMP-9) and MMP-2 were analysed by ELISA kits in gestational hypertension (GH), late onset preeclampsia (LOPE), early onset preeclampsia (EOPE), eclampsia (E) and control pregnant women (CPW) during third trimester. The Pregnancy outcomes like the gestational age at the time of delivery (GA) and birthweight (BW) and APGAR score of the baby also were measured. In the study the GA, BW, APGAR score of the baby were found to be significantly lower in EOPE and Eclampsia compared to CPW. The circulating levels of angiogenic factors, PLGF, TGF-b were significantly lower in EOPE and Eclampsia compared to CPW, GH. Besides, the antiangiogenic factors sVEGFR1 and sEng were found to be elevated in Preeclampsia-Eclampsia when compared to CPW. A significant correlation was observed between the plasma angiogenic factors and the pregnancy outcome in HDP. The study concluded that an impaired angiogenic profile and a poor birth outcome were observed in hypertensive disorders of pregnancy.

P250

Thyroid Autoimmunity and Perinatal Outcome

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Data regarding effect of maternal thyroid dysfunction on offspring are inconsistent. The objective of the study was to investigate the effects of thyroid dysfunction or antibody positivity on perinatal outcome. Two hundred pregnant women with singlet pregnancy (gestational age between 6^{th} to 14^{th} weeks) were selected and grouped according to TSH levels: Group 1 (Control, n = 100): TSH levels between 0 to 2.5 mIU/L with normal FT_3& FT_4, Group 2 (Test, n = 100): TSH levels >2.5 mIU/L with normal FT_3& FT_4. Baseline thyroid function tests (TSH, FT_3, FT_4, TPO Ab and TG Ab) were done. The patients were followed till delivery for perinatal outcome. Maternal and cord blood TSH were collected at the time of delivery. TSH, FT_3, FT_4, TPO Ab and TG Ab were done by competitive immunoassay using direct chemiluminescence technology. In Group 1, all the patients were thyroid auto-antibody negative. TPOAb positivity was 14% and TGAb positivity was 7% in group 2. Complications were more in TG Ab negative patients as compared to TPO Ab positive patients. Subclinical hypothyroidism and thyroid antibody positivity were associated with increased incidence of low birth weight babies. The study concluded that first-trimester antibody positivity is a risk factor for perinatal death. Thyroid autoantibodies have more diagnostic potential in giving clues for adverse perinatal outcome as compared to thyroid hormone titre alone. It is advisable that thyroid autoantibodies must be tested in risk pregnancies.

P251

Sepsis in Pre-term Neonates

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Sepsis is a potentially life-threatening complication of an infection which has increasing incidence among hospitalised patients. Sepsis occurs when some chemicals trigger inflammatory responses throughout the body, causing multiple organ system failure. Many

studies have shown that tumour necrosis factor (TNF) is the prime mediator of the inflammatory response seen in sepsis and septic shock. IL-4 is a key regulator in humoral and adaptive immunity. Daily measurement of CRP is useful as it is one of the most sensitive sepsis markers. The aim of the study was to correlation of TNF-alpha and IL-4 with CRP in the saliva of pre-term neonates. Sepsis is one of the leading causes of preterm mortality. Diagnosis at an early period after birth improves chances of survival. Hospital bound diseases like sepsis requires immediate, close attention and monitoring. A non-invasive technique involving body fluids easily accessible like saliva can make the identification process less painful. Since sepsis is majorly triggered by infection, identification of key components of our immune system becomes important. The objectives of the study were analysis and correlation of TNF-alpha, IL-4 and CRP in saliva of preterm neonates, usage of non-invasive procedure and identifying the marker for the diagnosis of sepsis in neonates. 100 saliva samples were collected from preterm neonates over a period of time using 2 cc sterile swabs which were used to swab the insides of the buccal cavity from which the saliva was then pumped into sterile Eppendorf tubes and measured for TNF-alpha, IL-4and CRP. It was stored at −20°C for processing. The sample was processed for TNF-alpha and IL-4 using ELISA kits. CRP was estimated turbid metrically.

P252

Gender-Based Differences in Cord Blood Lipid Profile

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A fetus needs considerable amount of cholesterol for the development of tissues and organs. After birth human lipid transport system gets transformed from low VLDL and LDL levels to the adult system containing high LDL levels that continue to increase with age. Elevation in LDL cholesterol levels in young adults have been linked with cardiovascular disease in later life. The present study was designed to analyze cord blood lipoproteins and to compare them in male and females. Study group was comprised of 100 healthy newborn following normal term delivery of healthy normotensive pregnant women. Hundred healthy volunteers served as controls. Lipid profile and Apo lipoproteins (A-1 and B) were analyzed in their serum and atherogenic index (A.I.) (ApoB/Apo-A1) was calculated. The study showed lipid profile (total cholesterol, triglyceride, VLDL, LDL and HDL cholesterol) levels were significantly lowered in cord blood as compared to adults. Total cholesterol, HDL-C, LDL-C, Apo A-1 and Apo-B were higher in female newborns as compared to male newborns. Whereas triglyceride and VLDL-C were higher in male newborns. In adults, lipid profile was higher in females as compared to males. Apo A-1 and Apo-B and A.I. were higher in adult males as compared to females. The study concluded that the lipid levels are influenced by gender-based factors. In utero environment plays a critical role in the development of adult disease and it is possible that fetal programming is sex specific. Also, atherogenic mileu is different in males and females since in utero.

P253

First Trimester Biomarkers in Prediction of Pregnancy Hypertension

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Hypertension in pregnancy is one of the potential causes of maternal and fetal morbidity and mortality. It complicates 7 - 10% of pregnancies Early interventions can improve the perinatal outcome. The objective of the study was evaluation of plasma associated pregnancy protein-A (PAPP-A), Free β human chorionic gonadotropin (Freeβ-hCG), tumor necrosis factor-α (TNF-α) and interferon gamma (INF-γ) in first trimester for establishing a biomarker or combination of biomarkers for the early identification of pregnancy hypertension. This study was carried out in two phases. Phase I was a prospective cohort study in which 1287 pregnant women were enrolled in their first trimester (gestation $11 + {}^{0}-13 + {}^{6}$weeks) and followed till delivery. Maternal variables such as body mass index (BMI) and mean arterial pressure (MAP) were recorded. Serum levels of placental markers such as PAPP-A and Free β-hCG were analysed by chemiluminscence. Women who developed hypertension were taken as cases and compared with women who remained normotensive throughout their pregnancy. In phase II we have done a case control study. The women who developed hypertension in phase I were cases and their gestational age and sample storage time matched controls were selected in which additional proinflammatory markers tumor necrosis factor-α (TNF-α) and interferon gamma (INF-γ) were analysed by ELISA. Appropriate stastical tests were used. In the study out of 1287 we have excluded 174 women (62: lost to follow up, 112: resulted in other adverse outcome). There were 129 women who developed hypertension in which 69 (5.4%) cases of Gestational hypertension (GHTN), 57 (4.6%) of Preeclampsia (PE), 3 (0.2%) of eclampsia (E). There were 984 women with normal outcome who remained normotensive throughout their pregnancy and gave birth to a healthy neonate. MAP ($p < 0.001$) and BMI ($p = 0.005$) were found significantly higher in hypertensive women when compared with control. Maternal serum levels of PAPP-A ($p < 0.001$) were significantly low in cases as compared to controls, while free β- hCG($p = 0.39$) was high in cases but the difference was not statistically significant. TNF-α ($p < 0.001$) and INF-γ ($p = 0.005$) both were high in cases. Increased levels of pro-inflammatory cytokines suggest the role of underlying inflammation in pathogenesis of pregnancy hypertension and low PAPP-A may be attributed to impaired implantation. Combining biomarkers may improve the prediction of pregnancy hypertension in the early pregnancy.

P254

Correlation of Gestational Lipid Profile with Birth Weight of Neonates

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Fetal growth is a complex process involving the interaction of mother, placenta, and fetus. Substantial evidence exists for clear relationship between lipid profile and neonatal birth weight. The objective of the study was to assess the status of lipid profile in pregnant women and its correlation with the birth weight of neonates. Present analytical study of 100 Indian pregnant women (without history of hypertension, diabetes and other endocrine disorders) was carried out at SMIMER medical college and hospital, Surat. Blood samples were taken from each woman for biochemical analysis who was supposed to undergo in labor within 24 hours of admission. The serum samples were analyzed immediately for the estimation of Total Cholesterol by using CHOD-PAP method, TG by GPO enzymatic method and HDL by Mg – phosphotungstic acid precipitation. For the estimation of LDL in mg/dl Friedwall's formula was used. The weight of each baby was done at the time of delivery. Differences were considered significant at p values of ≤ 0.05. In the study positive correlation was found between maternal lipid profile (Except HDL) and neonatal birth weight. The maternal HDL and neonatal birth weight showed no any correlation. The study concluded that maternal lipid profile should be kept under observation to control the weight of the newborn baby.

P255

Arterial Blood Gas Alterations in Head and Neck Surgery under General Endotracheal Anaesthesia with Relation to Cardiopulmonary Changes

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General anaesthesia is a controlled state of unconsciousness achieved with a group of drugs. The surgical procedure, the anaesthesia, the supine position etc., are expected to affect the respiratory physiology, resulting in ventilation perfusion mismatch, hence alterations in Arterial Blood Gas (ABG) parameters. Though a known fact, yet no study has exactly quantified these changes, whose detection might help to modify the post-anaesthetic care as per the condition as well as enable us to prevent any post-operative complications. The objective of the study was to measure the alterations in cardiopulmonary and ABG parameters in head and neck surgery under general endotracheal anaesthesia and their relation with duration of anaesthetic procedure. A pilot study of 25 patients of 18–60 yrs, ASA class I& II during head and neck surgery under general endotracheal anaesthesia were included in the study after getting written consent. Patients with uncontrolled coagulopathies, modified Allen's Test negative and surgery in prone position were excluded from the study. Pre-operative cardiopulmonary assessment along with ABG analysis was performed as baseline values. All patients were administered and monitored with standardised anaesthetic procedure till recovery. Another two samples for ABG analysis were collected at recovery and 24hrs after recovery. Clinically significant alterations in ABG parameters were noticed at recovery and 24hrs post-operatively after recovery compared to baseline values, being related to duration of surgery. The study concluded that the early detection of ABG changes may help in assessment of normal regain of respiratory function and encourages for O_2-administration and ventilator support in indicated cases without exposing all patients to deleterious effects of O_2-therapy and ventilator support.

P256

Effect of Medicinal Plant Extract (*Ocimum sanctum)* and Functional Food Extracts (*Zingiber officinale, Piper nigrum*) on Gut Beneficial Bacteria

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The present study explored the prebiotic potential of *Ocimum sanctum, Zingiber officinale* and *Piper nigrum,* which are extensively used in Ayurveda for treating immune inflammatory diseases. The Objective of the study was to test the prebiotic potential of *Ocimum sanctum, Zingiber officinale* and *Piper nigrum* using gut beneficial bacteria Lactobacillus (*Lactobacillus rhamnosus* GG and *Bifidobacterium (Bifidobacterium infantis*). Preliminary phytochemical screening, antioxidant activity and HPLC analysis was carried out on the extracts of *Ocimum sanctum, Zingiber officinale* and *Piper nigrum*to determine the presence of active compounds. Prebiotic potential was carried out using *Lactobacillus* and *Bifidobacterium* bacteria by standard plate count method. Fructo-oligosaccharide was the standard prebiotic. In the study preliminary phytochemical screening showed the presence of tannins, flavonoids, alkaloids, saponins and phenolic compounds. *O. sanctum, Z. officinale* and *P. nigrum* showed a modest antioxidant activity when compared with ascorbic acid. *O. sanctum, Z. officinale* showed prebiotic activity at 2.5 ppm concentration with abundant growth of *Lactobacilli* and *Bifidobacteria,* whereas *P. nigrum* showed enhanced growth of *Lactobacilli* and *Bifidobacteria* at much higher concentration (25.0 ppm) similarly to Fructo-oligosaccharide. The study concluded that the prebiotic activity of these herbs could be due to the presence of phytochemicals. Prebiotic potential of these herbs may be exploited to regulate gut bacteria, which in turn will prevent systemic inflammation and associated disorders.

P257

Role of Nitric Oxide Donor and Ciprofloxacin Against Typhoid

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Typhoid caused by *Salmonella typhi* remains a major health concern worldwide. The emergence of multidrug-resistant (MDR) strains of Salmonella with increased virulence leading to increased morbidity and mortality has further complicated its management. Human typhoid is similar to the infection caused by *Salmonella typhimurium* in mice. Most of the antibiotic are resistant and vaccines have less than desired efficacy and certain unacceptable side effects, making it pertinent to search for new suitable formulation. Nitric oxide (NO) is a gaseous free radical molecule produced in biological systems. During enzymatic conversion of L- arginine to L-citrulline by NO synthase (NOS) nitric oxide is produced. Ciprofloxacin one such fluoroquinolones have been shown to achieve high intracellular concentrations and least resistant antibiotic used against typhoid. Exogenous administration of L - arginine results in increased NO production, indicating that endogenous substrate is insufficient for maximal NO production. By considering these facts, it was thought to see the effect of oral administration of NO donor i.e. L arginine along with the low doses of antibiotic (ciprofloxacin). NO estimation was done by the fluorometric method Misko et al, (1993) with slight modification. In the study hepatic nitrite level in mice infected with 0.6xLD50 of *S. typhimurium* was 8.33%, higher than control animals (treated with saline) at day 8, and in group B + Arg, B + Cip & B + 1/2Arg + 1/2Cip were 16.66%, and 12.5% & 10.25% respectively as compared to only *S. typhimurium* infected mice. Formulation of L-arginine and ciprofloxacin shows better therapeutic induction against typhoid. The study concludes this increase of nitrite level (metabolites of nitric oxide) may be due to enhanced cytokine expression.

P258

Viral Load Monitoring by Real Time PCR and its Clinical Usage

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Hepatitis is a disease of liver that causes its inflammation and potentially may result in permanent damage. Hepatitis B virus (HBV) infection continues to be a leading cause of chronic liver disease. The measurement of HBV DNA in serum can not only help to monitor treatment efficacy but also indicates infection rate and drug resistance emergence. The present study includes 44 HBsAg positive chronic hepatitis B patients to quantify the viral load. This study included 44 randomly selected patients with chronic hepatitis. All the serum specimens were collected from the Department of Gastroenterology and Internal Medicine, Shri Mahant Indiresh Hospital, Dehradun which includes both indoor and outdoor patients. Patients confirming anti HBV antibodies utilizing third generation ELISA kit from Erba diagnostics Mannheium, Germany were further processed for HBV DNA quantification which was done by Roche Taqman 48 Real-Time Polymerase Chain Reaction (RT-PCR). In the study 44 cases undergoing antiviral treatment were processed for HBV DNA viral load. These patients carried HBV DNA titer in between 6.00×10^2 to 1.75×10^8. Out of 44 patients, 4 were negative in which the target DNA was not detected. It signifies their complete remedial procedure. The study concluded that advances in the molecular diagnosis of viral diseases using highly sensitive methodologies such as DNA amplification by RT-PCR can further detect upcoming viral resistance at an early stage when the variant represents only a minor fraction of the total viral population. Such new tools are especially relevant for patients at high risk for disease progression or acute exacerbation.

P259

Morus alba Linn: Multi functional Tonic for Life

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The importance of Ayurveda by naturally occurring plants is being revitalized to alleviate the diseases without harming the body. *Morus alba* is a medicinal plant originating from Asia but currently cultivated in subtropical, tropical, and moderate environments. The Phytochemicals (alkaloids, polyphenols, flavonoids, and anthocyanins) are one of the best naturally occurring tonic. Phytochemical could provide protection against free radical production, degenerative diseases, Atherosclerosis, cancer, Parkinson's, Alzheimers, Diabetes mellitus but still the role of active principle compounds should be unveiled. The objective of the present study was to identify phytochemicals in methanolic extracts of *Morus alba* (leaf, fruit and stem). Mulberry plant was collected from the Pune region of Maharashtra, India. The plant was identified and authenticated by Botanical Survey of India, Pune. The healthy plant parts (leaf, stem and fruit) were washed and dried at room temperature. The plant material extracted with analytical grade methanol (1gm powder in 5 ml of methanol) and extract was stored at 4°C until analysis. The extracts were used to identify the presence of phytochemicals using standard methods. Results showed that all screened phytochemicals are present in all three extracts (leaf, fruit and stem). They were; carbohydrate, phytosterol, cardiac glycoside, flavonoid, phenol, saponin, tannin and terpenoid. However quinone was absent in fruit extracts and present in leaf and stem extracts only. The study concluded that the presence of phytochemicals suggests that *Morus alba* could serve as a dietary source for treatment of various diseases. Further phytochemical studies are needed for various diseases.

P260

A Novel Electrochemical RNA Sensor for Quick Detection of H1N1 (Swine Flu) in Human

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Swine Flu (H1N1), an influenza A subtype, consist of negative sense single stranded RNA. H1N1 virus is famous for their property of antigenic shift and antigenic drift which makes their diagnosis difficult. Due to this the patients show severe complexities in disease as compared to other seasonal influenza subtypes. The available methods for the detection of swine flu are real time RT-PCR, conventional PCR and immunological methods having disadvantages like less sensitivity, specificity, false positive and false negative results, high cost and time consuming. The objective of the study was to develop gold composite based RNA sensor for quick, economical and early detection of swine flu. Screen printed gold electrode, redox indicator, coupling agents, specific probe and buffer are required for electrochemical reactions. Probe was fabricated on gold electrode for the early detection of H1N1. The HA gene based biosensor was developed by immobilization of 24 mer amine labeled ssDNA probe on the gold electrode. The immobilized probe was hybridized with different concentrations of complementary strands of probe and $_{C}$DNA of H1N1 from nasal swab. The electrochemical response was measured by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). In the study the lower limit of detection of the sensor was found 0.0014 ng from DPV measurements. The sensitivity of the sensor was found to be 113.968 μA cm^{-2} ng^{-1}. The total time for diagnosis of the disease takes only 30 min. Specific HA gene based probe was immobilized on gold screen printed electrode and electrochemical changes was measured by CV and DPV at different concentrations of complementary RNA strands. Specificity of the sensor was found only with H1N1 not other pathogens.

P261

Malondialdehyde and Reduced Glutathione in Neonatal seizures

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Seizures in neonates and young infants present a frequent diagnostic challenge. Incidence in the newborn baby is 1.5–3.5 per 1000 live term births, 10–130 per 1000 live preterm births. After exclusion of acquired causes, disturbances of the internal homeostasis and brain malformations, the physician must evaluate for inborn errors of metabolism and for other non-malformative genetic disorders as the cause of seizures. Reactive oxygen species have been implicated in the development of seizures under pathological conditions and linked to seizure-induced neuro degeneration. There has been little direct evidence, however, of free radical production resulting from the seizures. The concentration of free radical induced lipid peroxidation (Malondialdehyde) has shown to increase in seizures. Antioxidants (glutathione) prevented the rise in lipid peroxidase but did not arrest the development of seizures. Institutional Ethics Committee permission was obtained for carrying out this study. Total of 60 subjects were included in this study and after obtaining written consent, 1 ml of venous blood was collected and used for the estimation of reduced glutathione and Malondialdehyde using spectrophotometric method. Inclusion criteria for cases- neonates having seizures and inclusion criteria for controls-age matched healthy volunteers.

P262

Blood Transfusion Reaction Diagnosed by Rising Serum Creatinine Levels - A Case Report

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Blood transfusion reactions are diagnosed by their clinical manifestations and require to be managed urgently and aggressively. An unusual presentation of severe blood transfusion reaction presented where the clinical manifestations were mild, however the serum creatinine levels ordered for routine monitoring of the patient showed rising levels. This led to review of the patient and diagnosis of severe blood transfusion reaction which lead to acute renal failure. Patient was accordingly managed including series of haemodialysis and eventual recovery of patient.

P263

Streptococcus pneumoniae UGPase in Virulence: *In Silico* Analysis and *In Vitro* Validation of its Putative Inhibitor(s)

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Streptococcus pneumoniae (Pneumococcus) causes several life threatening diseases like meningitis, bacteremia, septicemia, otitis media, and pneumonia etc. The pneumococcal disease burden is fueled due to rise of new serotypes and spread of antimicrobial resistant clones. The available capsular-polysaccharide based vaccines remains unsatisfactory due to limited serotype protection, implementation issues and high economic burden upon developing countries. Hence, formulation of alternative prevention measures especially independent of serotypes is inevitable for better management of pneumococcal diseases. The Objective of the study is as in *S. pneumoniae,* the capsular biosynthetic pathway shares sugar-precursors with metabolic Leloir pathway i.e. nucleotide linked sugars. The

genes encoding for these precursors are common to all pneumococci regardless of capsular types. UDP-glucose is a fundamental precursor in both the pathways and synthesized in a reversible reaction catalyzed by the enzyme UDP-Glucosepyrophosphorylase (UGPase). Pneumococcal UGPase serves as logical checkpoint for committed glucosyl residues in capsule synthesis and hence, present itself as a promising candidate to control pneumococcal virulence. The structure of pneumococcal UGPase was modeled using I-TASSERserver and the putative inhibitors were searched. The selected inhibitor was validated in vitro for its efficacy and safety. The activity of UGPase was evaluated spectrophotometrically in a coupled reaction at 340 nm in UGPase assay. Adherence of *S. pneumoniae* to A549 cells was evaluated in the presence and absence of inhibitor using flow cytometry. The toxicity of inhibitor to A549 cells was assessed using MTT assay. In the study I-TASSER server generated five 3D structure models of pneumococcalUGPase, out of which model one was predicted as most accurate form its C-score (1.72). The inhibitor (U1) was selected based on active site residues and its effective inhibitory concentration was evaluated from dose dependent inhibition of UGPase activity. The activity of pneumococcal UGPase was significantly inhibited at 5 μM concentration ($p < 0.05$) where as 20 times concentration of inhibitor was required to decrease the UGPase activity in A549 cells. During interaction with host, adherence of *S. pneumoniae* strains to A549 cells was effectively decreased in the presence of inhibitor ($p < 0.01$). Furthermore, Inhibitor didn't affect viability of A549cells though it lowered the percent cell cytotoxicity even in the presence of bacterial infection effective from 5 μM concentration ($p < 0.05$). The study concluded that *S. pneumoniae* UGPase inhibitor seems promising to lower pneumococcal virulence in vitro, without exerting any harmful effect on host cells. Further, in vivo UGPase inhibition studies would be beneficial for translating the findings of this study.

P264

Differential Proteome Analysis of Peripheral Blood Mononuclear Cells from Patients of Concurrent Active Tuberculosis and Type 2 Diabetes Mellitus

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Diabetes almost triples the risk of developing active tuberculosis and is also a risk factor for its adverse treatment outcomes. Eight of the ten countries with the highest incidence of diabetes mellitus worldwide are also classified as high-burden countries for tuberculosis by the World Health Organization. Though decreased immunity in diabetic patients is thought to be the cause for this co-occurrence, pathophysiology of association of tuberculosis and diabetes mellitus is poorly understood. Proteomics is an important tool which can be applied to understand the pathophysiology of concurrent cases of tuberculosis and diabetes. The objective of the study was to analyze the differential proteome of PBMCs in concurrent active tuberculosis and type 2 diabetes patients. Forty one candidates were enrolled in the study and were categorized in four different groups: i) patients with pulmonary naïve tuberculosis, ii) associated pulmonary naïve tuberculosis and type 2 diabetes, iii) only type 2 diabetes and iv) healthy controls. PBMC were isolated and subjected to proteome analysis 2DE-MALDI-MS/MS. Proteins were identified by MASCOT and specific functions for the proteins were correlated with SwissProt database. In the study a total of 272 ± 29 spots were obtained on the 2DE gel. Spot analysis was done by Image Master Pentium 6 software. Two or more fold change in spot area was considered significant for alteration in protein expression. A total of 24 proteins were found to have altered expression among the groups. Out of which Vimentin is highly expressed on *M. tuberculosis* infected human monocytes and is involved in binding to NKp46 receptors of NK cells. Another protein Maff-interacting protein that acts as transcription factor is involved in upregulated production of IL10 Hyaluronan synthase 1 in MDMs infected with *M. tuberculosis* which favours bacterial growth. The study concludes that involvement of these differentially expressed molecules in various cellular networks can be predicted which might be linked to the co pathogenesis of tuberculosis and diabetes.

P265

To Study the Correlation of hsCRP and Carotid Intima Medial Thickness with Severity of Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory disease that results in severe disability and premature mortality. Rheumatic inflammatory diseases confer increased risk of cardiovascular diseases. High sensitivity C-reactive protein (hsCRP) is a sensitive marker of systemic inflammation in RA and Carotid artery intima medial thickness (CIMT) is used to assess atherosclerotic burden and cardiovascular risk. The present study was conducted to study the association of hsCRP and CIMT in RA patients and to correlate these parameters with the severity of rheumatoid arthritis. The study was conducted in VMMC and SJH, New Delhi. Thirty diagnosed cases of RA based on Revised American College Of Rheumatology and the European League Against Rheumatism were taken up for study. Severity was measured in terms of DAS-28 score. B mode ultrasonography was used to measure the CIMT and hsCRP assay was performed using ELISA method. In the study the mean hsCRP value was significantly higher in cases (34.35 mg/l) as compared to control (4.4 mg/l). Although there was a rising trend in mean value of hsCRP with severity of RA but was statistically insignificant. CIMT was also found to be significantly higher in cases as compared to control and also showed significant correlation with the severity of RA. The study concludes that RA is an inflammatory disease and hsCRP is a marker of inflammation and its value is positively correlated with the severity of disease. Carotid artery intima media thickness has also significant correlation with severity of Rheumatoid arthritis. Hence, the early detection of hsCRP and CIMT can prevent morbidity and mortality due to cardiovascular events.

P266

Acute Viral Myositis with Myoglobinuria

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Myositis characterized by pain, tenderness, swelling, and weakness of a voluntary group of muscles. The causes include autoimmune conditions, genetic disorders, medications, electrolyte imbalances, endocrine disorders, and infections. Infectious myositis occurs due to a wide variety of pathogens including bacteria, viruses, parasites, and fungi. Bacterial myositis presents as focal muscle infection, whereas viruses and parasites tend to cause diffuse disease with generalized myalgias and multifocal myositis. Viruses can induce myositis through production of immune complexes, immune dysregulation, or other mechanisms. The viruses commonly implicated include influenza A/B, parainfluenza, coxsackie, herpes simplex, Ebstein Barr, cytomegalovirus, dengue virus, chicken gunya and adenovirus. We have investigated a case of a 9 year old female was admitted to Kasturba Hospital, Manipal, Karnataka, India, with complaints of passing red colour urine as well as thigh and elbow pain for 1 day. In the study base line test for CPK was 457300 U/L and urine myoglobin protein was strongly positive and a fatty acid oxidation defect work up was done in this child which was normal. The study concludes that rhabdomyolysis can occur as a result of exertion, crush injuries, seizures, drug abuse, alcohol, viruses, and statin use. The diagnosis of viral myositis as a possible cause of rhabdomyolysis was suspected in this case based on clinical presentation and laboratory evaluations.

P267

Urea Cycle Disorder in Indian Population

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Urea cycle disorders (UCD) are a group of inborn errors of metabolism characterized by hyperammonemia, metabolic alkalosis and clinical features of encephalopathy. These are among the commonest type of inborn errors of metabolism with a frequency in 1 in 8,000 to 1 in 30,000 in different Indian population. Urea cycle disorder (UCD) resulting from mutations affecting removal of ammonia from the blood stream. This encompasses 5 major disorders, corresponding with at each step in urea cycle namely Ornithine trancarbamoylase (OTC) deficiency, Arginosuccinate lyase deficiency, Carbamoyl phosphate synthatase (CPS) deficiency, Citrullinemia and Argininemia. The most important clinical presentation is neurological abnormalities. The severity of UCD is correlated to extent of hyperammonemia with little effect from environment. UCD affects both Newborns as well as adults. Newborns with severe mutation becomes extreamly ill within 36–48 hours of birth. About 20% of sudden infant death syndrome (SIDS) cases may actually be due to an undiagnosed IEM, such as urea cycle disorder (UCD). Some mutations are very severe as they fail to produce any enzyme that needed to carry out nitrogen breakdown. Some mutations are mild to moderate which means that a certain amount of enzyme will be present and this would enable to detoxification of ammonia. Adult with suble symptoms go unnoticed or undiagnosed because they produce enough enzymes to adequately remove ammonia until there is an interference with the enzyme function to be produced. Usually this interference is brought about by metabolic stressors including viruses, excessive dieting or exercising, high protein intake or certain drugs such as valporic acid, prednisone or other corticosteroids. UCD can be diagnosed by quantitative analysis of plasma amino acids and analysis of urine Orotic acid through the technique/method using LC-MS/MS and GC-MS. In presently in our Genetics Department we diagnosed 25 cases of urea cycle disorder in both newborn and adults. There are no cures for UCD however the condition can be remedied through liver transplant. We will present urea cycle disorders detected both in pediatric population and in adults.

P268

Study of Serum Calcium in Maternal and Cord Blood of Women with Preeclampsia and Normotensive Pregnancies

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The aim of this study wasto assess Serum Calcium (SCa) levels in the women with preeclamptic and normotensive pregnancies and to estimate its levels in cord blood with a view to relate the levels in pregnant mothers with their new born babies. This observational study was carried out in the Department of Biochemistry in PGIMS, Rohtak from February 2015 to July 2015. Thirty apparently normal pregnant women and 30 preeclamptic pregnant mothers were enrolled in the study. Subjects were excluded if they had chronic medical disease or were taking medications known to interfere with Ca metabolism such as corticosteroids, thyroxine and heparin. Total SCa, Ionized calcium (Ca + 2), corrected total Ca and serum albumin (Alb) were estimated in pregnant females. There was a highly significant reduction in total SCa and corrected total Ca in preeclamptic pregnancies when compared to normotensive pregnancies ($P < 0.001$, $P < 0.001$ respectively). Serum Ca + 2 also showed significant difference in these two groups ($P < 0.02$). The significant difference in serum Alb levels were noticed in normotensive and preeclamptic subjects. ($P < 0.001$). Total Ca level in cord blood of normotensive was statistically highly significant when compared to cord blood total Ca levels inpreeclamptic pregnancies ($P < 0.001$). In our study, there was a strong positive correlation in total SCa levels in cord blood of newborn and in serum of both healthy and preeclamptic mothers and was statistically highly significant ($r = 0.562$, $r = 0.680$ and $p < 0.001$, $p < 0.001$ respectively). We conclude from our study that reduction in maternal total SCa with consequent decrease in Ca + 2 as a fraction of total calcium, may have role in development of preeclampsia. Low SCain preeclamptic mothers and in cord blood of their babies could be a useful indicator of the maternal and fetal complications. This biochemical marker would allow early identification of patients at risk of preeclampsia and thus help in providing adequate prenatal care and reduce the maternal mortality.

P269

Iron Status in Pediatric Patients with Beta Thalassemia and Sickle Cell Disease

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Blood transfusion is the main stay of supportive treatment in patients with thalassemia and sickle cell disease which is life saving at the same time can cause overt side effects. The aim of the study was to investigate iron status in regularly transfused patients with thalassemia and sickle cell disease. This comparative study was carried out in the Pediatrics Department and lab investigations being carried out in the clinical biochemistry laboratory and RIA centre for one year. 50 cases of thalassemia and 30 cases of sickle cell disease were enrolled for study. Serum iron and TIBC estimated in semiautoanalyzer by colorimetric method and ferritin estimated by radiometric immunoassay method. In patients with thalassemia mean level of iron, TIBC and Ferritin found to be 194.24 ± 23.67 μg/dl, 240.62 ± 35.78 μg/dl and 1220.3 ± 411.61 ng/ml while in sickle cell disease patients results were 165.23 ± 19.77 μg/dl, 262.83 ± 27.12 μg/dl and 656.67 ± 373.04 ng/ml respectively. Iron overload seen in both thalassemia and sickle cell disease patients who are regularly transfused also iron and ferritin has positive correlation with number of blood transfusion and results were statistically significant. From these results it can be concluded that estimation of iron status is important in transfusion dependant patients as management for iron overload and its untoward effect can be done at the earliest with proper monitoring.

P270

Induction of *rec*A in *E. coli* Using Sub-lethal Concentrations of Replication Inhibitors Mitomycin C and Nalidixic Acid

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Targeted gene replacement in bacterial chromosome occurs at very low frequency and depends on host homologous recombination. The key mediator of this process is recombinase A whose expression is induced when there is DNA damage. The efficiency of this process may be improved by increasing the expression of *rec*A gene, which is known to be induced in response to replication inhibitors. In this study we explored the use of mitomycin C (MMC) and nalidixic acid (Nal), two known replication inhibitors, for determining the optimal sub-lethal concentrations of these agents for inducing *rec*A expression. The aim was to study the induction of *rec*A expression in *E. coli* in response to a range of sub-lethal concentrations of the replication inhibitors mitomycin C and nalidixic acid. We used *E. coli* K-12 strain for this study. At first the minimal inhibitory concentrations (MIC) of MMC and Nal were determined. Bacterial cultures grown to mid log phase were treated for 1 hour with a range of sub-lethal concentrations of MMC and Nal. Complementary DNA was prepared from the total RNA isolated from treated and untreated (control) cells for assessing the expression level of *rec*A by Quantitative RealTime PCR. For normalization between the samples 16S rRNA was also assayed. The MIC of MMC and Nal for *E. coli* K-12 were found to be 2 and 4 μg/mL respectively. The *rec*A expression was increased to 35, 8, 4 and 3 fold in presence of 0.1, 0.2, 0.5 and 1 μg/mL of MMC and 2, 20, and 65 fold in presence of 2, 4 and 6 μg/mL of Nalin comparison with the untreated bacteria. Both the replication inhibitors could bring about significant increase in*E. coli. recA* expression. The decreasing trend of *rec*A expression with increasing sub lethal concentration of MMC could not be explained at this juncture. However, as low as 0.1 μg/mL of MMC would suffice the purpose of improving the recombinational efficiency when targeted gene replacement has to be achieved.

P271

Use of the N-terminal 42 Amino acids of Holin as Candidate Lethal Peptide for Genetic Construction of Bacteria Capable of Conditional Suicide

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Holins are gene products encoded by the lytic bacteriophages and have a key function in the lysis of the bacterial cells by forming holes in the cell membrane. Holins consist of a membrane spanning N-terminal hydrophobic domain and a hydrophilic C-terminal domain. In lambda phage the 107 amino acid long Holin is encoded by the S gene which along with other gene products bring about bacterial lysis at the end of lytic cycle. The Lambda S gene regulation is highly complex involving two initiation codons and additional regulatory motifs which tightly restrict its translation until its presence is required. This study was initiated with the aim to exploit the Lambda S gene as the candidate lethal gene for genetic construction of bacteria capable of conditional suicide. We assembled the nucleotide sequence encoding Holin by recombinant PCR using 8 overlapping oligonucleotides. After confirmation of the nucleotide sequence the PCR product was cloned into pASK75 vector under the control of tetracycline (tet) regulatable promoter. The recombinant clones were initially checked for suicide phenotype upon induction with anhydro tetracycline. One of the clones which showed potent suicide phenotype was found to have a mutation resulting in the introduction of a stop codon after 42 amino acids. This truncated peptide is rich in hydrophobic aminoacids and has 5 cationic aminoacids as well. We further engineered this construct oftet inducible S gene encoding truncated but functional Holin for targeted integration into Escheriacoli K12 strain at the rec A locus. This strategy can be applied for the construction of live bacterial vaccine strains whose persistence in vaccinated individuals can be controlled by induction with a small dose of tetracycline. Conditional suicide phenotype can also be used for the containment of genetically engineered bacteria.

P272

Therapeutic Efficacy of D-penicillamine Nanoparticles in Rat Model of Non-Wilsonian Brain Copper Toxicosis

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Animal models of Wilson's disease (WD) viz. Long evans cinnamon rats, rarely exhibit neurological symptoms impeding the development of novel therapeutic approaches to treat neurological manifestationsin WD patients. The aim of this study was to examine the effect of intraperitoneally injected copper lactate for 90 days especially on copper and zinc levels in liver, kidney & brain tissues; expression of hepatic metallothionein-I (MT-I) and Atp7b gene; and MT-III and acetylcholine esterase (AChE) gene in brain, biochemical parameters, and neurobehavioral functions of male Wistar rats, and therapeutic evaluation of orally administered D-penicillamine encapsulated alginate/chitosan nanoparticles for 90 days on Cu intoxicated Wistar rats. Reverse transcription-PCR and Morris water maze test were used for expression and neurobehavioral studies. Copper intoxicated animals showed significantly increased ceruloplasmin, serum & urine copper levels and decreased serum acetyl choline esterase (AChE) activity, increased expression of hepatic MT-I gene with impaired neuromuscular coordination and spatial memory. However, no changes were observed on the expression levels of hepatic Atp7b gene; and MT-III and AChE gene in brain. Cu intoxicated rats revealed a significant increase in the liver, brain and kidney tissues copper content (99.1, 73 and 74.9 % increase respectively), decreased liver zinc (40% decrease) & interestingly, increased brain zinc content (77.1% increase) compared to controls rats. Histopathological studies demonstrated grade 4 copper depositions in the liver and grade 1 copper associated protein in liver tissues of test rats by rhodanine and orcein stains respectively. Astrocytes swelling were observed in cerebral cortex sections of brain tissues of Cu intoxicated rats. D-penicillamine encapsulated alginate/chitosan nanoparticles therapy resulted in significant reduction of liver and brain Cu content, serum ceruloplasmin level & increase in serum AChE activity with improvement in neurological functions. In conclusion, chronic copper toxicity may lead to increased copper content in liver & brain, increased hepatic MT-I gene expression, ceruloplasmin levels and, neurobehavioral impairments may be by interfering in acetylcholine modulated neurotransmission; however, D-penicillamine encapsulatednanoparticles may reverseimpairments caused by chronic Cu intoxication to a significant extentin Wistar rats.

P273

Duchenne Muscular Dystrophy in UT Chandigarh Patients: Analysis of Deletion Pattern in Dystrophin Gene Utilizing Multiplex PCR

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Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder that affects 1 in 3,600 - 6,000 males and is caused by mutation in the dystrophin gene. In this paper, we have reported DNA based diagnosis of DMD patients by multiplex polymerase chain reaction (M-PCR) from UT-Chandigarh, India. Genomic DNA was extracted from 500 µl of whole blood and characterized by M-PCR for 25 exons for deletions. Of the 45 clinically suspected patients of DMD, deletion was detected by M-PCR for 25 exons in 27 (60%) patients. Majority of the deletions 92.6% were located at distal hot spot region that encompasses exons 44–55 and 3.7% of the deletions were located at the proximal hot spot region (exons 2–19). In this study, the observed deletion frequency was 60% and was more frequent in the distal end exon. Mutation detection is evidently crucial for diagnosis but it may also be significant for future therapeutic purposes. Further research is important to elucidate specific mutation pattern in association with management and therapies of proband.

P274

Inhibition of Angiotensin converting Enzyme in Sheep (*Ovisaries)* Tissues from *Centellaasiatica*: An *in vitro* Study

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Angiotensin converting enzyme (EC: 3.4.15.1, ACE) catalyzes the hydrolysis of angiotensin I to angiotensin II. ACE and angiotensin II are biologically active components of renin-angiotensin-aldosterone system, which play central role in the maintenance of blood pressure, electrolyte and water homeostasis. ACE inhibitors are used in the treatment of congestive heart failure, coronary artery disease, diabetic nephropathy etc. Many medicinal plant active components are used as drugs for the treatment of high blood pressure. The present study was on inhibition of ACE activity in sheep (*Ovisaries*) tissues from extract of *Centellaasiatica*. Tissue ACE activity was measured with Hippuryl-Histidyl-leucine (HHL) as substrate and the hippuric acid released was measured at 228 nm. Methanol extract of *Centellaasiatica*was used in the enzyme assay to determine its effect on kidney, lung and testis ACE activity. The linearity of ACE activity of kidney, lung and testis enzyme was established for the incubation period of 30 min at 37°C. ACE activity was confirmed by Captopril, a known inhibitor of ACE. ACE activity was determined in the presence of methanol extract of *Centellaasiatica* (10:1), it inhibited ACE activity significantly. 25 µls of *C. asiatica* leaves extract reduced sheep kidney, lung and testis ACE activity by 45%, 59% and 43% respectively. Use of medicinal plant is gaining considerable importance by reducing blood pressure. Significant inhibition of kidney, lung and testis ACE activity by *Centellaasiatica* extract indicates that, it may be beneficial in controlling blood pressure or in cardiovascular diseases.

P275

Human Serum Albumin: Possible Effect on Microbiome and Bacterial Pigment Production

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Microbiome is one of the crucial factors for maintenance of physiology. It lives with a symbiotic association with the human body. Albumin is a protein made by liver. It maintains osmotic pressure and acts as a carrier for the transport of hormones, fatty acids and other compounds. Till now, the direct effect of albumin on microbial flora and bacterial production of pigments is not reported. The objective of this work is to study the effect of human serum albumin on some components of microbiome as well as pigment production of some bacteria known for pigment production. Bacterial strains were cultured under standard conditions with and without human serum albumin and checked for growth and pigment production. We have seen that human serum albumin affects the growth of several microorganisms. It is particularly observed that albumin enhances pigment production of some pathogenic strain of bacteria. The observations documented in this study hints that human serum albumin may have important role in our microbiome as well as in pigment production of some pathogenic bacterium.

P276

Silibinin Ameliorates Neurobehavioural and Mitochondrial Defects in Experimental Model of Huntington's Disease

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Huntington's disease (HD) is neurodegenerative disorder, genetically caused by pathological CAG-triplet repeat extension(s), within the gene product, huntingtin (IT15 gene), is characterized by progressive motor dysfunction, involuntary abnormal choreiform movements etc. Mechanisms of cell death are unclear, but mutant huntingtin aggregation, mitochondrial oxidative stress, dysfunctions and decreased activity of electron transport chain complexes have been implicated in pathogenesis of HD by various studies. 3-Nitropropionic acid (3-NP) is a neurotoxin which induces mitochondrial dysfunction by covalently binding and inactivating active site complex II of electron transport chain, thereby increasing striatal neurotoxicity that mimic the diseased phenotype in non-human primates and animal models. Promising approaches have been provided by neuroprotective antioxidants (curcumin, lycopene, quercetin, N-acetyl-cysteine, ginko biloba, flavonoids, etc.) in diminishing progression of HD. Silibinin is a plant derived herbal product shown to have antioxidant, chemopreventive, hepatoprotective, and neuroprotective effects and it have been utilised as potential therapeutic agent for the treating neurodegenerative disease like Alzheimer's disease. Hence silibinin have been chosen as the drug of choice for in vivo study after evaluating its effect in vitro experiments. The objective of study was to investigate the neuroprotective benefits of most effective flavonoid (silibinin) from in-vitro study against 3-nitropropionic acid (3-NP) induced animal model of HD. *In vitro* study was designed to find the most effective out of six selected flavonoids in reducing the lipid peroxidation and increasing the reduction of MTT. For in-vivo study female wistar rats were taken and animal model of Huntington 's disease was induced by using 3-NP. Animals were segregated into four groups viz. Control (Vehicle), Silibinin, 3-NP, Silibinin + 3-NP. Each animal was analysed for motor co-ordination and gait through neurobehavioral studies, and mitochondrial respiratory chain enzymes, mitochondrial oxidative stress parameters. Under *in-vitro* conditions silibinin significantly reversed the effect of 3-NP induced lipid peroxidation and MTT reduction. Under *in-vivo* conditions, administration of silibinin significantly increased locomotor activity, glutathione, superoxide dismutase activity and weight of 3-NP treated animals. Supplementation of silibinin lowered malondialdehyde (MDA), reactive oxygen species (ROS), protein carbonyl and mitochondrial swelling in striatum of 3-NP treated animals. Moreover silibinin treated animals exhibited higher activities of mitochondrial complexes and cytochromes. Thus, the results of this study suggest that silibinin administration is beneficial in management of HD in animal model and might be a promising therapeutic intervention to ameliorate mitochondrial dysfunctions in this disease.

P277

Intracellular Trafficking of *Mycobacterium Tuberculosis* within Human Type II Alveolar Epithelial Cells

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Mycobacterium tuberculosis (*M. tb*) replicates and persists inside the host cells like macrophages. Other than professional phagocytes, alveolar epithelial cells (AECs) have also been reported as niche for intracellular survival of *M. tb*, thus contributing to pathogenesis of TB. However, fate of *M. tb* in endocytic pathway and role of host actin assembly for its survival in AECs is yet to be explored. Therefore, present study was designed to analyse the intracellular-trafficking of *M. tb* within AECs as compared to macrophages. Trafficking of *M. tb* in A549 cells and role of actin assembly was evaluated by studying co-localization of fluorescent M. *tb*H_{37}Rv with phagocytic markers and phalloidin staining using confocal microscopy. As compared to uninfected AECs, there was increased fluorescence for lysosomal markers and actin polymerization upon mycobacterial infection. Similar to macrophages, co-localisation of *M. tb* with endosomal markers (LAMP1/LAMP2) and phalloidin (polymerized actin) was observed 1, 2 and 3 days post infection in AECs. However, there was no co-localization of *M. tb* with Lysotracker red suggesting inhibition of acidification of phagosomes. Further data on the role of lysosomal enzymes in this pathway will also be presented. Similar to professional phagocytic cells, intracellular mycobacteria in AECs follow phagocytic pathway, also involving host actin assembly in the process. *M. tb* is able to survive within the phagolysosomal compartments of the AECs by preventing phagosomematuration thus escaping the lysosomal degradation.

P278

Mycobacterium Avium KatG-N (N-Terminal Region of Catalase-Peroxidase) Promotes Necrosis in Epithelial Cells

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Mycobacterium avium specific subcellular components which are cytotoxic to host might be responsible for the disseminating ability of *M. avium*. Virulent mycobacteria can escape innate host defense and spread by cellular necrosis. Earlier, our lab showed that KatG protein (catalase-peroxidase, 748 amino acids) isolated from *M. avium* culture filtrate was specifically reactive with sera from MAC patients. Also, first 40 a.a. region of its N-terminal (KatG-N) were only ~13% homologous to *M. tb* KatG-N. The study was aimed to elucidate the possible involvement of *M. avium* specific KatG-N in dissemination (the hallmark of opportunistic *M. avium* in AIDS patients) in alveolar (A549) and intestinal (HT-29) epithelial cells (representative of respiratory and gastrointestinal routes of *M. avium* acquisition). *M. avium* native KatG protein (748 a.a.) was first evaluated for its binding to A549 and then compared with *M. avium* KatG-N synthetic peptide (40 a.a) for effect on host cellular integrity. Mechanism of epithelial cytotoxicity observed with MTT assay was subsequently determined by acridine orange/ethidium bromide (AO/EB) staining, DNA fragmentation, LDH assay and annexin-V/PI staining. 20 μg/ml of *M. avium* KatG and KatG-N for 72 hrs reduced the cell viability of A549 cells to a similar degree (58.99% and 63% respectively). Further, EB uptake, LDH release, absence of DNA fragmentation and PI uptake suggested that *M. avium* KatG-N induced epithelial cytotoxicity was due to necrosis. Necrotic death of epithelial cells by *M. avium* KatG-N might contribute to *M. avium* dissemination across the epithelial barrier.

P279

Frequency of Inherited Metabolic Diseases (imds) in High-Risk Children in North India- PGI Experience

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Newborn screening, not commonplace in our country necessitates the shift to high risk screening. We present our data from a cohort in North India. The aim of the study was to study the frequency of various IMDs in high-risk children. A total of 1100 suspected cases with red flag signs suggestive of metabolic disorder were enrolled from March, 2012 to July, 2015 from various wards of the Advanced Pediatric Centre like pediatric ICU, neonatal ICU, emergency, OPD and neurodevelopment clinic. Children from age group 0–12 years were included. These patients were subjected to initial blood and urine screening tests followed by thin-layer chromatography, tandem mass spectrometry (dried blood spots), GCMS (urine samples) and HPLC (plasma and urine). Enzyme estimations were done for biotinidase and prolidase deficiencies. Majority of the children (638/1100) were in the age group of 1–12 years. Male to female ratio was 1.9:1. We were able to diagnose 10.4% children with IMDs. Sixty-three children had homocystinuria, out of which thirty three had combined homocystinuria and methylmalonic aciduria. Other disorders detected were glutaric aciduria (11), biotinidase deficiency (9), severe carnitine deficiency (9), methylmalonic acidemia (5), maple syrup urine disease (4), urea cycle defect (3), propionic acidemia (2), carnitine palmitoyl transferase-1 deficiency (2), succinic-semialdehyde dehydrogenase deficiency (2), congenital lactic acidosis (1), phenylketonuria (1), short-chain hydroxyacyl-CoA dehydrogenase deficiency (1) and prolidase deficiency (1). Amongst the investigated children, 10.4% were diagnosed with IMDs. The most common IMD was combined homocystinuria and methylmalonic aciduria. Implementation of a national newborn screening is of paramount importance.

P280

Development of A Protocol for Rapid Staining for Acetylcholinesterase: A quick Karnovsky Equivalent

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Acetylcholinesterase staining in tissues is clinically important for diagnosing Hirschsprung's disease. Such staining is otherwise required in an emergency set up to know the extent of aganglionic fragment before resection and anastomosis. Here we are reporting a rapid protocol for staining of acetylcholinesterase in haemolysate. Systematic in-silico and experimental observations are done to explore alternative substrate for acetylcholinesterase activity in comparison to acetylthiocholine that yields a stainable product. Importance of exploring alternative substrates for detecting acetylcholinesterase activity is described earlier. Our protocol has yielded rapid staining for erythrocyte acetylcholinesterase. Our developed method may be done with rectal biopsy sample to know its efficacy in emergency settings. It has the potential to detect acute pesticide poisoning at the point of care. The classical method for staining acetylcholinesterase is reported by Karnovsky and Roots. Several modifications of such method have been published till date. It is also a popular acetylcholinesterase staining method. However, still staining of acetylcholinesterase activity adopting Karnovsky and Roots method requires minimum 8 hours incubation. So this method is not suitable for emergency settings. If our developed method is proved to be valuable in clinical studies with a large number of samples, then it may overcome Karnovsky and Roots staining protocols limitation as far as staining time is concerned.

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A study of Acetylthiocholine hydrolysis at various pH

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Enzymatic hydrolysis of acetylthiocholine (ATCh) is measured to detect acetylcholinesterase (AChE) activity. It is commonly done to estimate RBC AChE activity in clinical biochemistry laboratories in the context of acute pesticide poisoning. However, non-enzymatic hydrolysis of ATCh is reported. This phenomenon imparts a high blank for assaying AChE activity. To the best of our knowledge, ATCh hydrolysis is not studied at various pH. We feel understanding of such fact is crucial for improvement of AChE assay procedure using ATCh. In this work we are reporting hydrolysis of ATCh at various pH. ATCh is prepared at various pH and checked for hydrolysis. ATCh hydrolysis is also checked in buffer prepared at different pH. It is observed that at basic pH ATCh is hydrolysed more rapidly which may cause a comparatively higher blank in assay procedures involving ATCh. It is also observed that ATCh prepared in distilled water produced lower blank. Spontaneous hydrolysis of ATCh is least in distilled water. Therefore, ATCh should be prepared in distilled water for assay of AChE activity.

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A Study of Serum Iron Profile and Total Antioxidant Status in Women Suffering from Breast Cancer

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Breast cancer is appearing to represent an enormous public health problem. The etiology of breast cancer involve interplay of genetic, hormonal and environmental factors that influence the physiological status of the host. Iron profile plays an important role in these patients. Iron is a double-edged sword catalyzing oxidative stress. Also low iron leads to VEGF formation, angiogenesis, metastasis and high recurrence. Ferritin is a major macromolecule that plays a role in iron storage and also an inflammatory marker. Antioxidant status of the body also altered in the patients.

The purpose of the study was to compare serum iron profile and total antioxidant status in women suffering from breast cancer with controls. Malwa includes south western districts of Punjab and cancer is more prone in this belt so our study was confined to Malwa belt of Punjab.

This study was conducted in the Department of Biochemistry and Surgery, G.G.S Medical College, Faridkot to find association with Iron profile and TAS in Breast Cancer. The study was conducted in 100 women diagnosed with breast cancer in the age group of 30-80 years and were compared with healthy controls.

Iron levels were found to be significantly lower in cancer patients as compared to controls ($p<0.01$). Serum IBCT and ferritin was found to be significantly higher in cancer patients as compared to controls ($p<0.05$). TAS was found to be significantly lower in breast cancer patients as compared to controls ($p<0.01$).

In the end we can conclude that though, iron does not have direct role in the pathophysiology of breast cancer but this parameter is relatively untouched and had been under looked in breast cancer and this study concludes that iron profile is deranged in breast cancer and may play important role in carcinoma breast along with antioxidant status of the patient and further research is required to support the study for better well being of the carcinoma breast patients.

P283

Altered mitochondrial function in pathogenesis of Systemic Lupus Erythematosus

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Systemic lupus erythematous (SLE), an autoimmune disorder, is associated with autoantibody synthesis and inflammation. Oxidative stress is one of the known contributors of inflammation. Oxygen is handled by the mitochondria, ''the powerhouse of the cell.'' Also, since major consumption of molecular oxygen takes place inside this organelle, they are also known to be the major producers of reactive oxygen intermediates in the cell. Mitochondria, the main producers of reactive-oxygen species (ROS), were studied to examine their role in the pathogenesis of SLE. Peripheral Blood Mononuclear Cells (PBMC) and mitochondria isolated from SLE patients (n=20) and healthy volunteers (n=20) were analysed for Mitochondrial ROS, swelling, hyperpolarization and levels of cytochrome c, caspase3. ROS was significantly increased in SLE patients (SLE vs controls: 1.83+1.03 vs 1.10+0.35; $p < 0.0001$) and more mitochondria from lupus samples were in depolarized state (SLE vs controls: 7.10+5.50% vs 2.5+1.8%; $p < 0.05$). Mitochondria swelling was found to be significantly altered in patients (SLE vs controls: 112.65+36.56 vs 60.49+20.69; $p < 0.001$). Expression of cytochrome c and caspase 3 (SLE vs controls: 1.37+0.37% vs 1.01+0.03%; 1.57+0.46% vs 1.06+0.07%; $p < 0.05$) respectively was found to be significantly increased in SLE. Further, the enzymatic activity of various mitochondrial complexes was assessed in isolated mitochondria. A significant decrease in activity of Complex I (SLE vs controls: 11.79+3.18 vs 15.10+6.38 nmol NADH oxidized/min/mg protein, $p < 0.05$); Complex IV (SLE vs control: 9.41+5.16 vs 13.56+5.92 nmol cytochrome c oxidized/min/mg protein, $p < 0.05$) and Complex V (SLE vs controls: 4.85+1.39 vs 6.17+2.02 nmol ATP hydrolyzed/min/mg protein, $p < 0.05$) was observed in SLE patients in comparison to healthy controls. However, Complex II did not show significant variation in either group (SLE vs controls: 42.2+28.6 vs

61.71+42.3 nmol succinate oxidized/min/mg protein; ns). The decrease in enzyme activities of mitochondrial Complexes I, IV and V on one hand and ROS, hyperpolarization and apoptosis on the other points toward a possible role of mitochondria in the pathogenesis of lupus.

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Derangements in Iron Homeostais in Inflammatory Bowel Disease

Molly Jacob

Anemia is a common feature in patients with inflammatory bowel disease (IBD). Adequate treatment of this condition is often a challenge. The pathogenesis of such anemia has been attributed to iron deficiency due to bleeding from the gut and to anemia of chronic disease (ACD). These conditions are characterized by marked derangements in iron metabolism. Current concepts of the processes involved in dysregulation of iron homeostasis in IBD will be reviewed.

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"Laboratory accreditation and quality control" - ISO 15189: 2012 Guidelines: NABL's Perspective

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Medical laboratory accreditation as per ISO 15189 across the globe is aimed at assuring "quality and competence". While ensuring quality of all resources across the entire quality management system is focused, special reference is made for the examination area ensuring the quality using external quality assurance for the patient safety.

The International standard ISO 15189:2012 has focused on the management responsibilities to assure quality system in the pre examination, examination and post examination processes. The need for the documented procedures at several of these processes has been the true guiding points for ensuring total quality management. With stress in the area of the duties and responsibilities and ethical aspects this standard has been a simple implementable prescriptive guideline helping the laboratory to geberate their own descriptive manual. This standard has made it easy to evaluate and document for the corrective action and eventually to prevent risk to the patients, Competence evaluation and the methodology adopted is well clarified.

Some of the highlights of this International standard is that it provided opportunities to the implementing agency in any country to respect and adopt the country specific requirement at both National and regional or local levels. The common fear about too much of documentation or the volumes of records is removed in his standard. This international standards has become a simple guiding point or the unique consultant to the laboratories to function ensuring the quality and competence and in the implementation total laboratory good practices.

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Apoprotein A1 as a biomarker for Coronary Artery Disease

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Dyslipidemias are known to associate with the risk and pathology of cardiovascular disorders. This notion along with observational evidences traditionally have recommended study of lipid profiles to assess the risk of cardiovascular disorders in a person or in a population. The metabolism of the endogenous and exogenous lipids is a function of the protein part of a lipoprotein, the apolipoproteins and, hence ,it is logical that time to time the importance of apolipoproteins as cardiac risk factors had been envisioned and studies had been undertaken. Over the years we had been working in this area.

We enrolled 500 patients diagnosed for CAD by the cardiologists at AIIMS, New Delhi and equal number of controls from the NCR region. The demographic details of the study subjects were collected using a questionnaire. A well designed exclusion and inclusion criteria was followed for the recruitment of the study subjects.

Lipid and lipoprotein profiles were determined and their association with CAD were evaluated. Patients were sub-grouped as those presenting with NCAD, SVD,DVD and TVD. The lipid, lipoprotein and selected apolipo-protein profiles were studied and their association with CAD was evaluated.

Of the lipid and lipo-protein parameters, only HDL emerged as the independent risk factor of CAD. Levels of apo A1 were lower in patients compared to controls, correlated with the severity of CAD. Our results suggested that the levels of A1, are genetically determined. In recent years, we took up a study on para-oxonase an HDL associated enzyme to evaluate its potential as a risk marker of CAD. The studies had been carried out both at the protein and gene level. Lower levels of arylesterase associated with the risk of CAD.

To conclude, while low HDL is an independent risk factor of CAD for the Asian Indians, levels of apo A1 may also be helpful in identifying the people at the risk of developing severe CAD. Association of paraoxonase with CAD needs further confirmation.

I express my heartfelt gratitude to Prof. LM Srivstava who initiated me in this area of research. The contribution of my students especially Dr. Shivani Chhabra and Dr. Imtiaz Ahmed is fondly acknowledged.

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The development of an Integrated Laboratory Management Training Program for Senior Health Laboratory Professionals

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Pathology and Laboratory Medicine is regarded as a complex service which needs sound business and operational principles for it to provide a well managed comprehensive diagnostic service. Many senior laboratory professionals, though academically sound, have little knowledge of strategic, operational or business planning. Many are also not aware of accreditation criteria or how to provide leadership in this complex environment. Increased attention to patient safety and awareness that laboratory results impact on patient treatment has made it a priority for laboratories to reduce errors and promote quality. Many laboratories in the developing world are in a dilapidated state, prompting organizations to attempt to improve the quality of results and strengthen capacity. Following a needs analysis as part of a strategic initiative to improve the management of laboratory services, we have developed an integrated laboratory management training program, which we believe is the first of its kind in Africa consisting of didactic lectures, practical sessions on strategic and business planning, an assignment, a project on clinical audits and an optional 12 week bench rotation in which the participants shadow the safety, quality and laboratory managers as well as rotate through the various disciplines of Laboratory Medicine. A key component of the program is focused on developing leadership skills and managing conflict. This course is now compulsory for doctors and scientists training in Pathology and Laboratory Medicine at Stellenbosch University, Cape Town. It has attracted senior laboratory professionals from Nigeria, Zambia, Ethiopia, Zimbabwe, UK, Kenya and has propelled laboratory training programs particularly in Nigeria, which has the most academic pathology laboratories in Africa. Strong support has also been received from the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). This program has also encouraged participants to publish their projects related to clinical audits in major international journals. Through the development of this program we have assisted the Royal College of Pathologists, UK and the College of Pathologists of East, Central and Southern Africa (COPECSA)to establish LabSkills Africa, which focuses on improving pre –analytical errors and laboratory interface in 5 African countries.

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Cardiometabolic Risk Factors and Abdominal Obesity: The Cholesterol of 21st Century

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Global cardiometabolic risk- is the overall risk of cardiovascular disease and type-2 diabetes. It includes traditional risk factors-such as smoking, hypertension and dyslipidemia and new emerging risk factors/markers caused mainly by abdominal obesity. These emerging metabolic abnormalities include insulin resistence, elevated apolipoprotein B, small dense LDL particles, reduced apolipoprotein A-1, a prothrombotic state and inflammation, All are part of a cluster of metabolic alterations often referred to as METABOLIC SYNDROME. When all features of metabolic syndrome are present simultaneously, they predict an increased risk of cardiovascular disease and type 2 diabetes. Possibly there is an intimate link between abdominal obesity and the metabolic syndrome and hence one must look beyond simple body weight.

Intra abdominal (visceral) obesity- which can be assessed through simple measurement of waist circumference-is perhaps the most serious new risk factor for cardiovascular and metabolic complcations and can be termed as CHOLESTEROL of 21st CENTURY. Excessive abdominal adipose tissue secretes a variety of biologically active substances that play a role in regulating lipid levels and insulin activityand contribute to the proinflammatory and prothrombotic state of coronary plaques leading to heart attack and stroke.

For these above reasons, physicians and patients need better, more indepth information about the health hazards of fat around waistline. Reducing this powerful cause of cardiometabolic morbi-mortality could have a significant positive impact on cardiometabolic diseases in general.

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Role of serum microRNAs 200a,200b and 200c as biomarkers in Epithelial Ovarian Cancer

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MicroRNAs have been implicated in the pathogenesis of different human cancers. Epithelial Ovarian Cancer (EOC), one of the most common and lethal gynecologic malignancies, is usually diagnosed at very late stages. Our aim was to investigate the role of three microRNAs in serum, belonging to the miR-200 family: miR 200a, miR 200b and miR 200c, in early diagnosis and prognostication of EOC.

We analysed the miRNA expression in serum samples from 70 EOC patients in comparison to control subjects. Total RNA was isolated from serum by Trizol method, polyadenylated and reverse transcribed into cDNA. Expression of miR-200a, miR-200b and miR-200c was measured by miRNA specific qRT-PCR, using RNU6B as a reference. Promoter hypermethylation of tumour suppressor genes BRCA1 and RASSF1A in DNA from serum of EOC patients was detected by Methylation Specific PCR (MSP).

Expression of miR-200a,miR-200b and miR200c in serum of EOC patients, was found to be greater than six-fold, three-fold and four-fold respectively, compared to age-matched healthy female volunteers ($p<0.0001$ for each miRNA). A significant correlation of promoter methylation of BRCA1 and RASSF1A genes with overexpression of miR200a /200b and miR200b/200c respectively, was observed.

Assesment by ROC curves of serum levels of these miRNAs for diagnosis of EOC, suggested a plausible role for only miR200a.Increased expression of miR-200b and miR-200c was significantly associated with advanced FIGO stage of EOC. Expression of miR-200a and miR200b was higher in the serous and mucinous subtypes of EOC. Patients with lymph node metastasis and distant metastases demonstrated significantly greater elevation of serum miR-200c ($p=0.002$ and < 0.0001 respectively). The overall survival of EOC patients decreased with escalating levels of miR-200a, miR200b and miR200c ($p=0.03$,0.008 and 0.03 respectively).

Our findings suggest that miR-200a, miR-200b and miR-200c overexpression may promote an aggressive tumor phenotype and their serum levels may be utilized as reliable biomarkers to predict prognosis and survival of EOC patients.

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An array of human recombinant antibodies from naturally recovered individuals for inhibition of preS1-hepatocyte interaction

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Monoclonal antibodies against HBV preS1-region are best suited for immune-based neutralization of HBV since the preS1 region (21-47a.a.) of HBV contains the viral hepatocyte-binding domain crucial for its infection through attachment to the hNTCP (Sodium taurocholate cotransporting polypeptide); the HBV receptor on hepatocytes. Passive prophylaxis is advocated for accidental exposure to Hepatitis B through administration of HBIG. But, emergence of HBIG 'escape mutants' is a limiting factor in its long term use.

Neutralizing anti-preS1 (21-47a.a.) antibodies are present in serum of spontaneously recovered individuals. We aimed to isolate the natural repertoire of such antibodies and utilize it to develop a therapeutic platform for HBV neutralization.

We generated a phage-displayed scFv library using circulating lymphocytes from individuals naturally recovered from Hepatitis B and isolated antibodies to target the HBV-hNTCP interaction for viral inhibition. The neutralizing capacity of the antibodies was shown in surrogate flow cytometry and fluorescence microscopy based neutralization assays through demonstration of their ability to prevent preS1-hNTCP interaction in HepG2 cells stably expressing hNTCP (HepG2-hNTCP-C4 cells).

Four preS1 specific scFvs with markedly distinct sequences were isolated (from the constructed library) which recognized the blood-derived and recombinant preS1 containing antigens. Each scFv showed a discrete binding signature, interacting with different amino acids within the preS1-peptide region. These antibodies inhibited preS1-hepatocyte interaction individually and even better in combination.

Such a combination of potentially neutralizing recombinant antibodies with defined specificities could mutants.

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Luteinizing hormone-Follicle-stimulating hormone ratio as Innovative biochemical predictor of post-partum depression

Raji R, Soundravally R*, Prabhat Leena be used for preventing/managing HBV infections, including those by possible escape

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Post-partum depression (PPD) is a common psychological disorder, which affects the wellbeing of both mother and newborn. Postpartum depression is usually assessed on EPDS (Edinburgh Postnatal Depression Scale) score, which is more of a subjective test. Serum Estrogen and progestogen levels though directly associated with PPD, have highly fluctuating values that hamper their diagnostic use. Gonadotropins [Luteinizing Hormone (LH), (Follicle Stimulating Hormone (FSH)] are a much stable parameter to identify PPD.

This was a nested case-control study done on 205 subjects who delivered in the hospital and came for postpartum checkup at 6th week postpartum. Detailed structured questionnaire about the socio-demographic characteristics, obstetric history, and details of the current pregnancy were given to subjects. PPD was assessed by EPDS. Based on EPDS score we divided the subjects into two groups, i.e., healthy subjects and PPD patients. Serum levels of FSH and LH were measured using direct competitive immunoassay by chemiluminescent. Spearman correlation analysis was used to assess the linear relation between serum LH, FSH and LH/FSH ratio with EPDS score.

We found significantly($p=0.02$) low levels of LH/FSH ratio in postpartum depression patients in comparison to normal postpartum subjects. We also found a significant correlation between LH/FSH ratio and EPDS. Moreover, we found significant ($p=0.006$) difference of LH/FSH ratio among subjects with different levels of postprandial depression as assessed using EPDS score. Further, we tried to establish the social and psychological effects of post-partum depression on the overall wellbeing of mother and newborn.

LH/FSH ratio can be used as a biochemical predictor of post-partum depression.