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Plenary

Proteomic Approaches and Potential of Clinical Applications: Glioma Perspective

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Mass spectrometry based Proteomics started in the new millennium for the time in India at the Center for Cellular and Molecular Biology, Hyderabad and we had the opportunity to venture into it then and still continue our efforts, although from another institute. Initially, the 2 D MS approach was our work horse and that of many other labs. With that initial effort, we were discussing some 20 and odd differentially expressed proteins being observed in multiple tumors, many of them major cytoplasmic proteins. Looking back today, we know we were exploring the proteome in a limited way, given the complexity of this tumor. So we changed our approach subsequently, moved to LC MS based quantitative proteomics and high resolution mass spectrometry and used all experimental systems like clinical tissues, plasma samples, glioma cell lines and decided to build a comprehensive data resource of molecular differentials observable in these tumors. Proteomic data can be further mapped to expression data at the transcript level from other public resources or to chromosome level changes in tumors. We have this annotated data resource of differentially regulated proteins. The talk will give an over view of this differential molecular repertoire, the range of annotations, the pathways and processes enriched, their secretory potential and leads from multiomics integration to develop clinical application relevant to these tumors.

Awadhesh Saran Memorial Oration

Human Papillomavirus and Cervical Cancer: Perspective and Prospect

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Epidemiology and molecular studies have confirmed the involvement of high risk HPVs especially HPV-16 and -18 as etiological agents in the development of cervical cancer and its pre-malignant lesions. Cervical cancer is the most common cancer in women in India because of poverty, lack of education and cancer awareness, coupled with absence of proper screening programs, late presentation by the patient and sub-optimal treatment. Indian accounts for 1,32,082 new cases, with mortality of 74,118 cases per year. Assessment of the prevalence and type distribution of HPVs and their variants across healthy controls and cervical cancer subjects as well as a cost effective screening test and therapeutic vaccine in India is needed.We have evaluated the type specific incidence of HPV infection among young married normal healthy women in the age group of 16-24 years. Exfoliated cervical cells were collected from 1238 normal subjects and tested for HPV types by PCR, Hybrid capture 2 and reverse line blot. The women were followed for 24 months, at 6 months intervals. Amongst the high risk HPV types, HPV 16 showed highest incidence rate, followed by HPV-59, -52 and -18 and all high risk types showed persistence, whereas for the low risk HPV types, HPV-42, -62, -84 and -89 were persistent. Region wise distribution of HPVs in biopsy samples from squamous cell carcinomas of the cervix was studied from different regions of India. Overall viral prevalence was 92.1% with HPV-16 and -18 constituting 79.6%. Infection with single high risk type was seen in 86.8% cases. We also identified HPV-16, L1, E6 and E7 variants associated with cervical cancer. The variants were characterized by full length sequence analysis of HPV 16 L1, E6 and E7. Similar distribution of HPV16 variants were seen from different regions of India with European variants E350G being the most prevalent (58%) followed by American Asian variant (11.4%). The most frequent changes in E6 region was L83V (72.3%), in the E7 region it was F57V (9%) and in L1 region they were 448 ins S (100%), 465 del D (100%), H228D (94%) and T292A (85%). The identified variants can disrupt pentamer formation, transcriptional regulation, B & T cell epitopes and p53 degradation. Knowledge of HPV incidence and type and variant distribution is important for development of HPV diagnostics, vaccine and for therapeutic purpose.The host genetic factors such as folate, homocysteine, cobalamin levels and methylation status of tumor suppressor genes in cervical cancer subjects were evaluated by ELISA in blood samples collected from 30 controls, 30 SILs and 30 cervical cancer subjects. Both SILs and cervical cancer subjects showed a decrease in folate levels, the decrease being more in case of cervical cancer. Low levels of serum folate and vitamin B12, whereas increased levels of homocysteine were observed in cervical cancer subjects. The three tumor suppressor genes studied i.e. CDH1, H1C1 and RAR-β displayed an increased frequency of promoter methylation, with increasing severity of cervical pathogenesis. Thus, poor folate and vitamin B12 status may contribute to cervical cancer risk through effects on one carbon metabolism and DNA methylation. Supplementation with folate and vitamin B12 could be a viable non-vaccine approach for prevention of cervical cancer in India. A gene methylation signature in women with low grade cervical neoplasm, holds promise as possible biomarkers of cervical cancer risk assessment. We also evaluated the polymorphism in DNA repair genes in cervical cancer pathogenesis. Deficiency of DNA repair system due to muations/polymorphism is associated with tumor progression. Folate is directly involved in DNA synthesis, DNA methylation and DNA repair via one-carbon metabolism, and its

deficiency has been implicated in the development of cervical cancer. DNA repair genes of base excision repair and nucleotide excision repair pathways were studied by PCR-RFLP and DNA sequencing. Maximum alterations were seen in XRCC1 (Codon 194 > 399 > 280) followed by ERCC4 (415 codon). DNA repair pathways may play a role through their repair, redox, folate deficiency in cervical cancer pathogenesis/progression. Prophylactic vaccines derived from the capsid protein of HPV16 and 18 are available commercially. However, therapeutic vaccines capable of eliminating HPV infection are not available. Hence, we designed strategies to develop vaccine which would have both prophylactic and therapeutic potential against HPV16. For formation of chimeric VLPs DNA was isolated from HPV16 positive cervical cancer sample, cloning of HPV 16 L1 and E7 was done in appropriate vector, recombinant protein was expressed in E.Coli and purified and assembled in vitro into chimeric VLPs. Immune response to cVLPs was measured by T cell proliferation assay, cytokines measured by ELISA, cytotoxicity using LDH assay and in vivo tumor induction and regression analysis done using TC-1 cells in C57 BL/6J mice. Subcutaneous injection with cVLPs enhanced serum IgG levels, showed lymphocyte proliferation, generated specific CTL mediated cytotoxic secretion of different cytokines. CTL generation in vitro against E7 protein essentially showed Th-1 response. Seroreactivity of young women with persistent HPV16 infection showed that the cVLPs detected neutralizing antibodies against L1 capsid protein. Immunodominant synthetic peptides having MHC class I- binding and CTL inducing epitopes from HPV16 viral L1, L2, E6 and E7 regions were designed by algorithm prediction software, were synthesized commercially having purity of $\geq$ 95% by HPLC. Microspheres were prepared using PLGA and the peptides encapsulated in them. C57BL/6J mice were immunized s.c. with these peptides. There was enhancement in serum IgG levels by 2.54 folds and lymphocyte proliferation by 2 fold. The CTL mediated cytotoxicity was 50.6% and there was increase in cytokine secretion of IFN α, IL2, IL4 & IL10. A 63.8% tumor regression was seen in mice. The findings provided a basis for rational design of peptide based vaccine and protein based chimeric vaccine for controlling HPV16 infection and its associated tumors.

K.L. Gupta Memorial Oration

Cancer Control Program and Screening of Cancer in India

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Cancer is a leading cause of morbidity and mortality in world. Every year 14.1 million new people in world are diagnosed and 8.2 million of them die due to cancer. More than half of this population is residing in developing countries like India. Every year 1 million new patients are diagnosed with cancer in India and are projected to increase by 40% by 2020. Tobacco is responsible for 42% of cancers in India. India started the National Cancer Control Program in 1976, joined IARC in 2006 and included cancer in the National Program for prevention of Cancer, Diabetes, Cardiovascular Disease and Stroke (NPCDCS). The program has goals of primary and secondary prevention, strengthening of treatment and palliative care facilities.The National Cancer Registry Program (NCRP) was initiated in 1982 by Indian Council of Medical Research, which gives the magnitude and patterns of cancer. There are 21 population based, 6 hospital based registries and several medical colleges from which data is being captured and available thorough internet as the *cancer atlas in India*.Visual inspection of cervix and oral cavity, tobacco control and IEC activities are feasible population based screening and preventive strategies for reducing the incidence of cancer with low resources like in India. The three main modalities of cancer treatment are surgical, radiation and medical oncology. More number of trained surgical oncologistsare required. 52% cancer patients need radiation therapy with 40% asa curative and others as a palliative treatment. The availability and utilization of morphine is an important goal for reducing the burden of pain in patients receiving palliative care.

Dr. Taranath Shetty Memorial Oration Popular Lecture

K V R Tagore

Director General of Police (Retd.), Bangalore, India

Mrs & Dr. G. P. Talwar Oration

Autoimmune Diseases: Therapeutic Role of Helminth Derived Immuno-modulatory Molecules

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Helminth parasites known to survive many years in human host are believed to be involved in offering protectionagainst autoimmune and allergic disorders. The *'hygiene hypothesis'* thusexplains the higher prevalence of various autoimmune and allergic disorders like inflammatory bowel Disease (IBD), Type 1 diabetes (T1D), Rheumatoid arthritis (RA), asthma etc.,in areas having reduced exposure to infections. Helminth infections associated with a strong Th2/Treg immune response are possibly able to modulate hosts' immunity by suppressing host-protective pro-inflammatory responses. Such immunomodulatory effects have been experimentally shown to have therapeutic implications in autoimmune disorders. In our lab, filarial parasite derived immunomodulators cystatin (rBmCys), abundant larval transcript -2 (rBmALT-2), and a RAL family protein (rWbL2) have been tested

for their therapeutic activity against T1D, colitis and RA. BALB/c mice treated with these proteins either before or after induction of T1D brought down the fasting blood glucose levels and the incidence of diabetes significantly (compared to untreated group) associated with reduced infiltration of pancreatic islets and histopathological changes. Mice treated with these proteins either before or after induction of ulcerative colitis also showed significant reduction in their weight loss, disease activity index, mucosal edema along with decreased myeloperoxidase activity and histopathological score in colon tissues. Substantial reduction in the mean articular index, histopathological score and inflammatory cytokines was also observed in mice that were treated with these proteins either prior to or after induction of arthritis. These results provide strong experimental evidence for using immunomodulatory molecules of helminthes as novel therapeutic agents for autoimmune diseases.

Dr. T. N. Pattabiraman Oration

Cutting the extra flab from your Lab platter: Lean-Six Sigma protocol holds the key

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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 defects per millionopportunities and express the value as sigma rating. In the analyze phase, tools used are root cause analysis. In the improve phase, we use decision analysis matrix. In the control phase, the tools are control charts, audits. Lean-six sigma helped us in the elimination of non-valueadded (NVA) steps and focusing on the value added (VA) steps. We have seen that 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 TAT, in August, 2011, we have 74.7% compliance and 3.14 Sigma. In November, 2013 it has improved to 99.3% compliance and 5.63 Sigma. Lean-Six sigmaensures that accurate and precise results are reported in a clinically relevant turnaround time.

Seth G. S. Medical College & KEM Hospital Oration

One Carbon Metabolism and Fetal Growth

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Indian children's body composition (Low Birth Weight, adiposity) predisposes them to Insulin Resistant Syndrome and Cardio-vascular-disease. Mechanisms involved in the impaired fetal growth and the long term consequences to the offspring caused by perturbation in the maternal one-carbon metabolism have been explored in the Pune-Maternal Nutrition Study. Maternal protein restriction and changes in the micronutrient status has a profound impact on one-carbon metabolism. Pune Maternal Nutritional Study (PMNS), has information on preconception maternal size and her nutrition and metabolism in pregnancy, fetal growth and birth size. The provision of unique information; determinants of fetal growth (thin Indian babies and adults have high adiposity, higher risk of diabetes and CVD), children born small and grown big have higher risk of DM and CVD, maternal vitamin B-12 and folate (one-carbon metabolism regulators) during pregnancy influences adiposity, insulin resistance in the offspring and the same influences cognitive function in the offspring have acknowledged the role of maternal nutrition. The study has confirmed that at critical stages in development, nutrient and environmental, influence through their effect on methyl transfer, reprogram metabolism and cause long term morbidity. The study has shown unique changes in the metabolism of methionine. The presentation will include: Metabolism of methionine, Metabolism of methionine in human pregnancy,Protein energy intake and methionine metabolism, one carbon metabolism and fetal growth and the role of homocysteine, folate and vitamin B-12.

S001

IFCC VLP
Interpretation of Clinical Laboratory Testing: Reference Intervals and Critical Values

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Clinical laboratory reports are required to include values other than the patient results to allow interpretation for the individual patient. Such reference values may arise from two strategies. Reference intervals are derived from a reference population that is usually healthy and comparable to the patient for important physiological characteristics such characteristics are dependent on the analyte. Comparison of a patient's value with such a reference interval can answer the question "Is this value usually seen in a healthy subject?" A major limitation of the reference interval strategy arises from biological variation both within the individual and across the population. For many clinically relevant analytes the individual biological variation is significantly smaller than the population biological variation. Therefore comparison of a patient's result with the reference interval can lack sensitivity such as when a level is outside of the healthy range for an individual but remains within the population healthy range. It is very difficult to determine healthy reference ranges for each individual before the present to the clinical laboratory for analyses. An alternative strategy is to define values which are associated with significant risk of disease. These are known as Critical Values. Comparison of the patient's value with a Critical Level can answer the question "Is this value usually associated with significant disease?" This strategy requires a much greater level of knowledge of the disease process than that required for the development of Reference Intervals. Both strategies are in current clinical practice and have their specific strengths and weaknesses depending on the analyte and disease process being monitored.

S002

IFCC VLP
Tools for Translational Medicine

Paolo Fortina

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Described as an effort to carry scientific knowledge "*from bench to bedside*", translational medicine builds on basic research advances to develop new devices and methods for diagnostics and treatment options for patients. Advances in technologies such as genomics and proteomics have improved our understanding of disease and provide ways to evaluate and treat diseases. Integration of these tools into clinical practice will speed up disease diagnosis, enhance treatment options, ultimately leading to improved clinical outcomes. As the technologies improve, key questions for their use become which ones to use and how best to their implement use. The objective of this talk is to provide up-to-date coverage of some of the emerging developments and technologies in the field of molecular medicine. Application of array-based gene expression profiling towards direct digital readout of each mRNA and their relative abundance and massively parallel DNA sequencing applied to single cell analysis will be addressed. Finally, the role of bioinformatics in disease diagnostics will be presented.

S003

IFCC VLP
Circulating Free Nucleic Acids: A New Tool for Molecular Diagnostics

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Advanced genetic diagnostics based on circulating molecular markers requires innovative methods for the detection of minority mutant alleles. This is particularly true in the case of mixed samples, where mutations are present at a low concentration among a background of wild-type sequences. The presence of fetal DNA in maternal plasma represents a source of genetic material which can be obtained non-invasively. To date, the translation of noninvasive prenatal diagnosis from research into clinical practice has been rather fragmented, and despite the advances in improving the analytical sensitivity of methods, distinguishing between fetal and maternal sequences remains very challenging. Thus, the field of noninvasive prenatal diagnosis of genetic diseases has yet to attain a routine application in clinical diagnostics. On the contrary, fetal sex determination in pregnancies at high risk of sex-linked disorders, tests for fetal RHD genotyping and non-invasive assessment of chromosomal aneuploidies are now available worldwide. Most of the molecular alterations found in cfDNA circulating in plasma reflect the genetic and epigenetic changes found in primary tumors and, thus, the analysis of such tumor cfDNA might be valuable for tumor diagnosis and monitoring. Highly sensitive methods are required to detect those alterations among larger quantities of non-altered cfDNA molecules. The clinical value of cfDNAs circulating in plasma is already more than a theoretical idea, since the characterization and the quantitation of such nucleic acids (NAs) have been shown to be complementary tools. It is therefore expected that in the coming years, an improved understanding of the relationship between CNAPS and the molecular biology of cancer will lead to better diagnosis, management, and treatment.

S004

Neurogenetic Disorders

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No other system in our human body is so widely affected by genetic conditions as nervous system. The neurological disorders not only baffled the neurologist but are also intriguing the experts in genetic conditions. To a general physician what may appear to be a normal variation may be an indicator of an early clinical presentation of a late onset neurogenetic disease. The clinical presentation of a genetic neurological condition may present with clumsiness to behavior problems to frank manifestation of abnormal movements. While clinical phenotype delineation can only be done by an experienced neurologist, the further management of the condition may bewilder even an expert. On the other hand a genetic expert may have wide experience of making a laboratory diagnosis of ever increasing genetic disorders with an armamentarium of genetic tools, he may never come close to provide support to family or community unless precise diagnosis established. Between the two extremes, we may provide working solutions which are not only useful for the patients but also helpful for establishing public health policies for some of these devastating conditions. In effect, this means embarking on an integrated approach for managing such conditions and extending the same to community incorporating into existing public health systems. To simplify the process, step wise approach may be needed with continuous dialogues between clinical biochemist and clinician. There is a need to create awareness about common neurological disorders, their genetic basis and available resources. Basic concepts of genetic counseling, genetic testing, types of diagnostic tests, their optimal utilization and interpretation is important. An environmental condition may mimick a disorder to be genetic. On the other hand a genetic neurological condition may occur due to different mutations located on different chromosomes. Recent advances in approaches to common genetic disorders like epilepsies, dementia, ataxia and movement disorders will be highlighted.

S005

Diagnostic Approach to a Patient with Storage Disorder

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Storage disorders are a group of metabolic disorders associated with the accumulation of metabolites in body cells and presenting with a chronic clinical course of myriad types. These disorders can be classified based on the tissue, cellular & subcellular location of the stored metabolites and on basis of the clinical presentation. The two major groups of storage disorders seen commonly in clinical practice are the Glycogen storage diseases and the large group of Lysosomal storage disorders. While the GSDs present primarily with glycogen accumulation with liver and/or muscle and an energy deficiency state, the LSDs have neurological and/or visceral involvement due to accumulation of large variety of distinctive metabolites for each disorder. The disorders with primarily visceral involvement present with hepatosplenomegaly and skeletal abnormalities. On the other hand neurological involvement is in the form of white matter or grey matter abnormalities of the central nervous system and presents as developmental delay or neuroregression. The clinical and laboratory diagnostic approach to patients with storage disorders will be discussed with illustrative case presentations.

S006

STR Based Prenatal Diagnosis for Aneuploidies by QF-PCR

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The term "aneuploidy" refers to a condition in which chromosome number is not an exact multiple of the usually haploid number. Aneuploidy is very common and clinically important, as they are associated with malformation and intellectual disability. Though many of these are spontaneously aborted, some are compatible with live birth, trisomy 21; trisomy 18; 47XXY and 47XXX. In general, among newborns, 0.3% of liveborns are aneuploid – the most common aneupoloidy is trisomy of 21 and sex chromosomes; however, in stillbirths, the incidence goes up to approximately 4%. Rapid aneuploidy testing (RAT) has been introduced in mid1990's in the form of fluorescence in situ hybridization (FISH) where testing can be done on uncultured amniocytes. Within a couple of years, multiplex ligation dependent amplification (MLPA) and quantitative fluorescent PCR (QF-PCR) has been added in the list of RAT. The other optional method of RAT includes: loss of heterozygosity assay (LOH assay), microarrary analysis, and automated DNA cytometry and, more recently, next generation sequencing (NGS). However, these methods are not commercialized for aneuploidy diagnosis due to their running cost, labor intensive protocol and complex data analysis. Among the rapid aneuploidy detection methods, QF-PCR has now become an alternative tool for prenatal aneuploidy diagnosis concomitant with karyotyping. This method has been validated in many of the western clinics but in India no study was conducted to assess its utility as standalone procedure. The study

was designed to answer the question whether QF-PCR can be implemented as a standalone diagnostic method for rapid aneuploidy diagnosis in our present clinical setup? Study was conducted during March 2012 to August 2014 consisting of 270 prenatal samples that underwent for aneuploidy diagnosis. Pregnant women's with positive indications for aneuploidy by biochemical screening or soft markers underwent invasive procedure as a routine prenatal diagnosis. In addition to karyotyping, QF-PCR was also performed on these samples and the results were compared. We included 5 markers – D21S1411; D21S11; D21S1411; D21S1435 and D21S1412 – for chromosome 21; 3 markers – D13S631, D13S634 and D13S258 – for chromosome 13; 4 markers – D18S535, D18S391, D18S499 and D18S51 – for chromosome 18; for sex chromosomes 7 markers – AMEL, SRY, XHPRT, DX6809, DX8377, TAF9L and X22 – were included. TAF9L has been included in the list with the purpose of indentifying monosomy of X chromosome. Primer will amplify paralogus sequence present in chromosome 3(136bp) and X (140bp), 1:1 and 2:1 ratio represents two and single copy of X chromosome, respectively. These markers were categorized into three sets, set A set B, set C and set D, based on their product size and fluorescent labeling. Of 270 samples screened, 262 samples showed euploid genome (125 normal male and 137 normal female). Eight samples were consistent with aneuploidy – four trisomy 21 male sample, 2 trisomy 21 female sample, 1 trisomy 18 samples and 1 Klinefelter sample. The specificity, sensitivity, positive prediction value and negative prediction values were 100% while false positive rate and false negative rate were 0%. Outcome of the present study strongly suggests that QF-PCR can be used as standalone procedure for targeted rapid aneuploidy diagnosis.

S007

Micro-array Technology: Role in Diagnosis of Intellectual Disability

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Mental retardation (MR) or intellectual Disability (ID) is a common problem in children. It poses a life-time burden on the patient, family and the society at large. ID can occur due to many different causes, both acquired and genetic. Multiple congenital anomalies (MCA) are also common in early childhood, with an incidence of approximately 3% at birth. Co-existence of the two significantly reduces the quality of life and increases morbidity and mortality in the affected children. Earlier, birth asphyxia, trauma and central nervous system infections were the major causes of ID in children. As the standard of pediatric care improved, the proportion of acquired causes has come down significantly. However, ID is still found in 1-3% of general population in India. When present before 5 year of age, it is termed as global developmental delay (GDD). In children, GDD is often associated with multiple congenital anomalies (MCA) or dysmorphism. These are indicators of underlying genetic mechanisms as a cause of mental subnormality. In others, there is no apparent cause. While managing these patients, conventional karyotyping or fluorescent in-situ hybridization (FISH)are performed to look for chromosomal anomalies. When the cause of ID is a known chromosomal anomaly e.g. Down syndrome, these tests confirm the diagnosis. Dilemma arises when there is no known phenotype into which the patient can fit. The conventional chromosomal analysis provides an answer only in 2-3% of the so called 'idiopathic' cases and other tests e.g. FISH and MLPA (multiplex ligation probe amplification), in even less.The trauma of having an unknown disease in the child takes a heavy financial, social and emotional toll on the family. Development of molecular karyotyping (MK) that can detect chromosomal aberrations of much smaller sizes and its application to GDD/ ID/MCA has been a significant achievement. The microarray technology has greatly increased the resolution of genome from megabases to kilobases in sub-microscopic deletions or duplications. It is observed that application of this technology to genome wide search for copy number variants (CNVs) and single nucleotide polymorphisms (SNPs) has yielded results in 10-25% of patients who were otherwise labelled as having MR/ID of unknown aetiology. Available evidence strongly supports the use of CMA in place of G-banded karyotyping as the first-tier cytogenetic diagnostic test for patients with DD/IDor MCA. Understanding the cause of their child's illness and the anticipated associated morbidities, even though not curable, permits the parents to plan management of the patient and make informed future reproductive choices.

S008

Thalassemia in India: From Bench to Bedside

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Thalassemia is a common genetic disorder and clinically more significant is beta thalassemia, in which beta-chain synthesis is reduced. This is common in some ethnic groups especially Sindhis, Lohanas, Bhanusalis etc with carrier rates ranging from 3-17% in North India. In severe thalassemia or thalassemia major, the presentation is in infancy and transfusions are needed to sustain life. The diagnosis can be made by HPLC analysis or hemoglobin electrophoresis. On HPLC analysis,the findings are: a) homozygous beta-thalassemia: HbF> 20 %, HbA2< 3.5%; b) heterozygous beta thalassemia: HbF < 10%, HbA2> 3.5%. Molecular diagnosis is necessary especially if further prenatal diagnosis is needed in the family. Beta mutations are characterized as β++, β+, β0, on the basis of severity of mutations. Over 200 mutations in beta-globin gene identified. However, some common mutations are prevalent in different populations. Five common mutations:IVS 1-5 (G-C),

IVS 1-1 (G-T), FS 8/9 (+G), FS 41/42 (-CTTT), and 619 bp deletion, account for over 95% of mutations in thalassemia major in North India. In thalassemia intermedia, the phenotype is modified by different genetic modifiers including severity of the beta mutations, associated alpha gene deletions, and XmnI polymorphism. Moreover, the polymorphisms in other genes can have epigenetic effects on the presentation or risk of complications, or response to therapy. The treatment in thalassemia major is lifelong transfusions and iron chelation therapy. The alternative form of therapy is bone marrow stem cell transplantation. In thalassemia intermedia, some cases may benefit from hydroxyurea therapy. Thalassemia minor is the carrier state and may not require any treatment, but transfusions may be needed during pregnancy or other stressful situations. The iron chelation therapy for reducing the iron overload in the patients is given in form of subcutaneous infusions of desferrioxamine, or oral therapy with deferiprone, of deferasirox. Gene therapy trials are underway for thalassemia in some centers, and new chelation agents are also under research.

S009

Genetic Counseling for Inborn Errors of Metabolism

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Metabolic disorders, although rare individually, can be attributed to a large amount of morbidity and mortality in infants and young children. Recent advances in the diagnosis and treatment of inborn errors of metabolism have improved substantially the prognosis formany of these conditions. This makes it essential that the practicing clinician be familiar with the clinical presentation of these disorders. Metabolic disorders can be detected only by keeping a high degree of suspicion, in cases where clinical symptoms are not suggestive of any particular disorder. Many of the inborn errors of metabolism, including urea cycle defects, organic acidemias, and certain disorders of amino acid metabolism, present in the young infant with symptoms of an acute or chronic metabolic encephalopathy. Therefore, appropriate laboratory testing for metabolic disorders should be performed in any infant who exhibits these findings. Although sepsis may be the initial consideration in a neonate with these symptoms, inborn errors of metabolism should always be in the differential diagnosis, particularly in a full-term infant with no specific risk factors. Identification of a particular inborn error of metabolism in a family is important as it helps in accurate genetic counseling. Genetic counseling is the process by which the family is explained about the occurrence or recurrence of a genetic disease. Genetic counseling helps the family to deal with the disease in the child as well as plan for next pregnancy with accurate carrier screening and prenatal diagnosis. A practical approach to the genetic counseling of inborn errors of metabolism in children will be presented.

S010

Calcium Cycling in Heart Failure: Twenty-Five Years On!

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Heart failure has global implications as ithave a high mortality rate and prevalence. Heart failure is heralded by a decrease in myocardial contractility that results in an impairment of the heart's ability to pump blood to meet the circulatory demands of the body. Eventually contractility continues to decrease into end stage year failure. With end-stage heart failure the circulatory demands of a patient can no longer be met**.** Abnormal calcium cycling has been implicated as being pathogenic for heart failure and its progression often leading to cardiac transplantation. Increased intracellular calcium load negatively impacts the myofilaments, myocardial contractility, ion channels as well as mitochondrial function (energetics) and oxidative stress.Our objective was to cause a paradigm shift away from the use of inotropic agents that increase intracellular calcium load towards a more targeted approach at the gene level. We performed pre-clinical testing on human myocardial samples, small animal models as well as large animal models in order to validate our target and to show safety and efficacy of targeting SERCA2a (sarcoplasmic reticulum calcium ATPase). A clinical trial has been initiated "CUPID". SERCA2a is the only gene targeted therapy for heart failure that has shown efficacy as well as safety. Increasing SERCA2a activity appears to be safe and to improve clinical outcomes in patients with end stage heart failure.

S011

Genetic Polymorphisms in the Assessment of Cardiovascular Risk

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Multigene and multifactorial involvements in the pathophysiology and progression of cardiovascular disorders are well accepted. The genetic variance is considered as the major determinant of individual's susceptibility to these diseases and heterogeneity of their manifestations. Candidate gene approaches and gene analysis are two established approaches in finding out the risk genes that predispose an individual to these diseases. Many of the global markers of cardiovascular disorders do not associate as conclusively with these diseases in India. Differences partially

must be due to the fact that gene-environment interactions are different in people from different ethnic, geographical and socioeconomic background. We aimed at comparative evaluation of selected candidate genes as the susceptibility or risk markers of CAD for the people belonging to Delhi and surrounding areas. DNA was isolated from the whole blood, PCR-RFLP was used to identify the gene variants. The study revealed significant association of apo A1 MSP1, -75 GA, eNOS glu298asp, and paraoxonase Q192R polymorphisms with CAD. Most interesting observation had been that aPON1gene variant which had been considered protective in studies carried out in west, was found to be associated with the risk of CAD in our subjects. Need of the time is to collect data from all the studies in India, go for an extensive meta-analyses, tally the findings with GWAS studies, pick up the most frequent risk gene variants establish their causal relations and develop preventive or therapeutic strategies accordingly.

S012

Clinical Significance of Emerging Cardiovascular Biomarkers in the Diagnosis of Coronary Artery Disease (CAD)

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Interest in cardiovascular biomarkers in primary prevention has increased manifold because of the advances in genetic and molecular research. Also, the Indian population is particularly prone to CAD, especially premature CAD, and traditional risk factors have failed to explain this high incidence. Currently two strategies are being followed to improve the management of CAD-search for novel biomarkers and the use of combination of multiple biomarkers. This has lead to the emergence of several novel clinically useful biomarkers that have improved the diagnosis and treatment of CAD and also helped to identify patients susceptible to this disease. The newer biomarkers for CAD have been classified according to the varied pathophysiology of the disease. Some emerging biomarkers include (a) small dense LDL- marker of plaque formation (b) Lp-PLA2 as a vascular specific inflammatory marker (c) oxidized LDL and myeloperoxidase as markers of oxidative stress (d) heart type fatty acid binding protein (FABB) as a marker of myocyte injury and apoptosis (e) marker for myocyte stress- soluble ST2 and (f) Cystatin C as a marker with extra cardiac involvement. We studied the association of two novel risk biomarkers, cystatin C and small dense LDL (sdLDL) with the presence and severity of CAD in patients ≤45 years with normal kidney function. Cystatin C was significantly raised and mean LDL particle size significantly reduced in CAD patients as compared to controls. sdLDL was significantly associated with the severity of CAD, while cystatin C was not. Both cystatin C and sdLDL emerged as independent risk factors, however, of the two, sdLDL was a moresensitive predictor of CAD events. Hence, we suggest, that while assessing the risk of CAD in younger age group patients, measurement of cystatin C and sdLDL may be considered along with the traditional lipid tests. Thus, in future, the risk management for CAD may shift from a single marker to a "multimarker approach."

S013

High Sensitivity (hs) Troponin: A Blessing in Disguise?

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The Universal Definition of Acute Myocardial Infarction changed in 2000 making biomarkers, particularly Troponin, central with a recommended cut off of the 99th percentile of a normal population. However, Troponin assays available at the time, could not measure that level reliably i.e. with a coefficient of variation of less than 10%. This was the driver for manufacturers to develop more sensitive assays.Troponin assays with improved analytical sensitivity, enable clinicians to diagnose or exclude myocardial infarction more quickly. However, this higher sensitivity means that marginally elevated Troponin values are detectable in patients without MI, which may cause confusion. These clinical dilemmas will be discussed together with the interpretation of hs Troponin assays. hs Troponin also allows measurement of Troponin values within the reference range and definition of separate 99th centile values of men and women. The potential clinical value of this will also be discussed.

S014

Identification of Differentially Expressed Proteins and Metabolites in Coronary Artery Disease Patients

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Coronary Artery Disease (CAD) is one of the largest cause of mortality and morbidity worldwide including developing countries like India. According to WHO, almost seven million deaths occur yearly due to this disease. It is estimated that global cardiovascular death would increase from 17.1 million (in 2004) to 23.4 million by 2030 with CAD contributing a significant proportion. Since CAD is a complex disorder where both genetic and lifestyle (including dietary habits) contribute significantly, finding new potential markers holds its own clinical importance specifically in

efficient management of the disease. The classical risk factors in blood like total cholesterol, HDL, LDL etc. are routinely determined to assess the risk of CAD. However, various prospective studies using these classical markers of CAD did not show a high predictive significance for the disease. Thus, it is imperative to identify other markers preferably from the blood plasma since the indication of patho-physiological status is reflected in this connective tissue and most importantly due to its accessibility. In the present study using high throughput iTRAQ based relative quantitation of plasma proteome we intended to identify proteins that are differentially expressed in stable coronary artery disease patients. Using iTRAQ based quantitative LC-MS proteomics technique we identified 18 proteins to be differentially expressed in CAD cases. These proteins were further validated in a different sample set (20 case and 20 controls) using ELISA. Eight proteins were found to have significantly altered levels in CAD patients. These eight proteins obtained in the verification stage were then further validated in a larger number of samples (253 cases and 253 controls). We employed a multivariate logistic regression analysis adjusting for age, sex, diet, diabetes, hypertension, smoking and statin intake for all the samples. Our results indicate that a panel of 4 proteins (Apo A1, ApoA4, Apo C1 and albumin) along with diabetes and hypertension could account for about 88% of the CAD cases. We have also used metabolomics as a tool to identify metabolites that could be used as potential markers for coronary artery disease. For this, blood samples from 18 angiographically proven CAD cases and 18 controls were analyzed in this study. 100 µl of plasma was precipitated using 200 µl of acetonitrile. The supernatant was lyophilized, redissolved and subsequently fractionated by reverse-phase and HILIC based methods followed by high resolution ESI-MS. In the reverse phase 160 peaks and in HILIC based LC-MS, 155 peaks and 175 peaks were found significant (P -value<0.05 with 2 fold up and down regulation) in the negative and positive mode respectively. To identify the metabolites we used HMDB library and METLIN database with a tolerance of 30 ppm (mass) and ± 2 minutes of retention time and validated each of them by matching the MS/MS fragmentation pattern. Using this approach we have identified 25 differentially regulated metabolites some of which have been previously reported to be associated with cardiovascular events (like Lyso PC, PE) We have also identified some metabolites that might be playing significant role in various processes that are associated with cardiovascular diseases.

S015

Biomarkers of Cardiac Injury

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A biomarker is a substance used as an indicator of a biologic state which gives Accurate values on repeated measurements at reasonable cost, Must provide additional information and aid treatment. The diagnosis of Acute Myocardial Infarction (AMI) by WHO requires at least two of the following evidences. 1, History of chest pain 2, Evolutionary changes in ECG and 3, Elevation of serial cardiac enzyme and proteins referred as cardiac markers. Cardiac Biomarkers can be broadly classified as 1. Biomarkers of myocardial injury, 2. Biomarkers of inflammation and 3. Biomarkes in Cardiac stress. Biomarkers of myocardial injury: Cardiac Enzymes: In most of the patients AMI, during the window period the enzymes released from the damaged myocardial tissues cause the elevated serum levels. The enzymes are creatine kinase (CK or CPK), Lactate dehydrognace (LDH) and Aspartate amino transferase (AST or SGOT) and the isoenzymes of CK and LDH. Cardiac The Cardiac Troponins (cTn) complex comprise of three proteins, (troponin C, I and T). Troponin I and T have cardio specific isoforms not found in skeletal muscle, making for highly specific markers of myocardial damage. They are released from necrotic myocardium either ischemic or non ischemic. Myoglobin is a heme protein found in skeletal and cardiac muscle with its low molecular weight accounts for its early release profile: myoglobin typically rises 2-4 hours after onset of infarction, peaks at 6-12 hours, and returns to normal within 24-36 hours. Serial sampling every 1-2 hours can increase the sensitivity and specificity; a rise of 25-40% over 1-2 hours is strongly suggestive of acute MI. It has only 90% sensitivity for acute MI. Heart –fatty acid binding protein (h-FABP) is a novel small cytosolic protein that is abundant in the heart. It is highly cardiac-specific, but is also expressed at low concentrations in tissues outside the heart. After myocardial ischemic damage, h-FABP can be detected in the blood as early as 1-3 h after onset of chest pain, with peak values reached at 6-8 h and plasma levels returning to normal within 24-30 h. h-FABP's clinical diagnostic value is very limited in the presence of renal failure and skeletal muscle diseases. Ischemia Modified Albumin (IMA) IMA is a novel marker of ischemia that is produced when circulating serum albumin contacts ischemic heart tissues. IMA levels rise within minutes of transient ischemia, peak within 6 hours, and can remain elevated for as long as 12 hours and return to normal within 24 hours. Studies on the use of IMA in patients with chest pain in the ED found sensitivities that ranged from 71-98% and specificities of 45-65%, with a negative predictive value of 90-97% for ACS.A multimarker approach in one study, using a combination of ECG findings, TnT levels, and IMA levels, achieved a sensitivity of 95% for ACS,while a second study calculated that the combination of IMA, myoglobin, CK-MB, and TnI increased the sensitivity to 97% for detecting myocardial ischemia. However, IMA levels are also elevated in patients with cirrhosis, certain infections, and advanced cancer, which reduce the specificity of the assay.

S016

Vitamin D Toxicity- Myth or Reality

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Public concern over vitamin D deficiency has led to widespread use of over the counter (OTC) vitamin D supplements, containing up to 10,000 IU per dose (400IU=10μg). Overzealous use of such supplements can result in vitamin D toxicity. Infants are particularly vulnerable to toxicity associated with vitamin D overdose. In a recent study only about 50% of OTC pills and one-third of compounded pills met USP Convention standards for vitamin D content. Most clinical symptoms related to vitamin toxicity are manifestations of hypercalcemia and hypercalciuria. Other causes of vitamin D induced hypercalcemia include hyperparathyroidism, granulomatous malignancies like sarcoidosis and mutations in the CYP24A1 gene. The differential diagnosis of hypercalcemia should include iatrogenic and genetic etiologies. Two important biochemical pathways via which 25(OH)D and 1,25(OH)D are converted to inactive metabolites are C24-hydroxylation and C3-epimerization. Mutations in the CYP24A1 gene cause reduced serum 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) to 25-hydroxyvitamin D 25(OH)D ratio (<0.02), elevated serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), hypercalcemia, hypercalciuria and nephrolithiasis. Studies in infants have shown that C3 epimer of 25(OH)D (3EPI-25(OH)D) can contribute 9–61.1% of the total 25(OH)D. Therefore, measurements of parathyroid hormone (PTH) and vitamin D metabolites 25(OH)D, $1,25(OH)_2D$, 3-Epi-25-hydroxyvitamin D (3EPI-25(OH)D) and $24,25(OH)_2D$ are useful to investigate whether the underlying cause of vitamin D toxicity is iatrogenic versus genetic. Here, we report a case of vitamin D associated toxicity in a 4-month old female who was exclusively breast-fed and received an oral liquid vitamin D supplement at a dose significantly higher than recommended on the label. The vitamin D content of the supplement was threefold higher (6000 IU of D/drop) than listed on the label (2000IU) brand name. Due to overdosing and higher vitamin D content, the infant received ~50,000 IU/day for two months resulting in severe hypercalcemia and nephrocalcinosis. I will also review the relevant literature on vitamin D toxicity in presentation.

S017

Role of Inflammatory Factors and Altered Neurotransmitters in the Development of Hepatic Encephalopathy following Bile Duct Ligation

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Hepatic Encephalopathy (HE) is a serious central nervous system complication of liver failure characterized by various neurological symptoms. A growing body of evidence demonstrates that inflammatory mechanisms act synergistically with ammoniatoxicity causing alterations in neurotransmission, leading to neuropsychiatric problems. The present study was designed to study the role of inflammatory factors and neurotransmitters in the development of HE. Male Wistar rats were subjected to bile duct ligation (BDL) surgery. Development of animal model of HE was assessed by routine liver function tests,ammonia levels, collagen content staining along with ^{99m} Tc labelled mebrofenin hepatic biliary clearance test. Cognitive assessment in BDL rats exhibited a progressive decline in learning, memory formation, retrieval, exploration of novel environment along with a decrease in serotonin levels. BDL rats also showed a significant decline in the time spent on the rotating rod, increased foot faults with difficulties to cross the narrow beam walk which was accompanied by a global decrease in the dopamine content in the brain. BDL also resulted in mitochondrial respiratory chain dysfunctions leading to generation of bio-energetic defects along with an increase in lipid peroxidation and protein carbonyls. Moreover, BDL also resulted in an increase in the inflammatory factors such as IL-6, TNF and MCP-1 in different regions of brain along with liver and serum suggesting for a role in development of HE. Histopathological studies using hematoxylin–eosin (H & E),cresyl violet exhibited anatomical changes in terms of marked neuronal degeneration, wherein neurons appeared more pyknotic, condensed and damaged. Overall, attenuation of oxidative stress, inflammation and restoration of neurotransmitter levels can provide new strategies for the prevention of HE.

S018

Prevalence of Vitamin D Deficiency and Its Association with Thyroid Stimulating Hormones Levels in Indian Population: A Retrospective Study

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Vitamin D is not only important to regulate calcium but also acts as immunomodulator and impart in thyroid disorders. Vitamin D influence thyrocytes directly by attenuating thyrotropin (TSH)-stimulating iodide uptake and cell survival. Several studies have shown that patients with hypothyroidism i.e. increased TSH levels have low vitamin D levels which may lead to cause some of the bone disease associated with hypothyroidism. Apart from the several studies it still to be investigate the relationship between vitamin D and TSH level and its prevalence in Indian population. In present retrospective cross sectional study, a total of 2500 adults' data (aged 20 – 70 years, 1647 male and 853 female) were randomly selected, who visited Executive health checkup at Medanta The Medicity, Hospital during July 2013 to July 2014. The mean age of 52.95 ± 12.6 (SD) years of male and 50.84 ± 11.2 (SD) years of female. About 79.1% of subjects were insufficient Vitamin D levels (<30 ng/mL), and about 29.7% were vitamin D deficient (<10 ng/mL). However, there was no significant difference in vitamin D level were observed between male and female (P>0.05). Subjects having vitamin D insufficiency were negatively correlated with increased TSH levels (r= -0.156 in male and r= -0.185). Whereas, subject with vitamin D deficiency were also negatively correlate with increased TSH level r= -0.234 in male and r= -0.0245 in female. However, no significant difference in TSH level was observed between male and female (P> 0.05). Therefore, it may be concluded that low level of vitamin D in male and female was negatively associated with increased TSH level, suggesting the involvement of vitamin D in the pathogenesis of hypothyroidism.

S019

Improvement in CVD Risk Prediction in Indians: Role of Lipoprotein-associated Phospholipase A2

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Indians have been found to be at the highest risk from cardiovascular diseases in the world. Improving the calculation of risk has a high clinical value, both for predicting risk of cardiovascular disease and improving the goal of preventive measures. LDL-cholesterol is a major target in the guidelines for the prevention of CVD. However, LDL-cholesterol concentrations are insufficient to identify individuals with incident CVD events because ~50% of all CVD events occur in persons with normal or even low LDL-cholesterol concentrations. This has led to the hypothesis that other factors may be involved in the pathogenesis of CVD. Recent clinical studies showed that lipoprotein-associated phospholipase A2 (Lp-PLA2) is an independent risk factor for CVD. Data from numerous large population studies and systematic reviews consistently indicated a positive association between plasma Lp-PLA2 mass or activity and the risk for incident atherosclerotic events. We sought to correlate Lp-PLA2 levels with routine Lipid biomarkers (Triglyceride[TG], Total Cholesterol[CHOL], HDL, LDL) and to explore if Lp-PLA2 offered added clinical benefit in CVD risk stratification in selected healthy as well as in high risk Indian individuals. A preliminary report on the ongoing case-control study would be presented. Data of 320 subjects (133 normal and 187 with history of one or more risk factors including past CVD event) was analysed in this report. Lipid parameters (TG, CHOL, HDL and direct LDL)were measured by previously cited biochemistry method. Lp-PLA2 activity was determined in plasma by ELISA using the PLAC™ assay of diaDexus Inc, USA. The mean Lp-PLA2 level in the selected normal healthy Indian population was found to be 173 mmol/min/ml (F=154; M=177). We observed a weak correlation of Lp-PLA2 with LDL and CHOL (R^2=0.33;0.28 and 0.31;0.18 respectively) and almost no correlation with TG and HDL levels (R^2=0.001;0.0009 and 0.008;0.02 respectively) in logistic regression analysis amongst both normal and with risk individuals. Also, Lp-PLA2 could help reclassify 29% of normal (low risk) and 11% of at risk subjects into higher risk levels. As observed in Caucasian populations Lp-PLA2 seemed to be a predictor of CVD risk independent of conventional lipid biomarkers in selected Indian population and it can be used to improve risk prediction.

S020

Psoriasis and Atherosclerosis: Shared Pathogenesis and Diagnostic Approach

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Psoriasis patients are often susceptible to cardiovascular diseases (CVD), including atherosclerosis. Traditional biochemical and inflammatory biomarkers and diagnostic tools could detect atherosclerosis. Carotid intima-media thickness (CIMT) has recently been recognized as a non-invasive diagnostic tool for identification

of premature atherosclerosis.We evaluated serum lipid profile, apolipoprotein B/apolipoprotein A-I ratio (apoB/apoA-I ratio), inflammatory mediators (serum leptin, hsCRP) and oxidative stress parameters (MDA, reduced GSH) in 150 psoriasis patients and 150 age sex matched healthy controls in relation with CIMT of carotid artery. Carotid intima-media thickness and carotid plaques were simultaneously measured by carotid sonography. The severity of disease (PASI) in these patients was found to be significantly associated with increased cholesterol, LDL, MDA and serum leptin. Increased CIMT in these patients correlated significantly with duration of disease, apo B/apo A1 ratio, serum leptin and hsCRP levels. Thus we may conclude from the finding of study that apo B/apo A1 ratio along with measurement of CIMT can prove to be potential markers for identification of risk of premature atherosclerosis in patients of psoriasis.

S021

Relationship between HbA1C Levels Versus Biochemical Markers of Systemic Inflammation in Type-2 Diabetes

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In this era Type-2 Diabetes, once called non-insulin-dependent diabetes, is the most common form of diabetes, affecting majority of the Indian population and by the next decade a major percentage of the population will be affected. To elucidate the role of HbA1C in Diabetes patients along with IL-6, TNF-alpha, IL-1 and hs-CRP with the biochemical markers to find out that if a low grade of chronic inflammation precede the onset of type-2 Diabetes Mellitus. 100 diabetes patients along with 100 controls were selected for study who attended the AN Hospital, Vizag from May 2014. Blood samples were collected and were analysed by ERBA Chem-7 semi auto analyser. The following biochemical parameters like, diabetic profile, lipid profile, LFT along with HbA1C was performed. The pro-inflammatory cytokines were done by ELISA method. Our results indicated that diabetic group had significantly higher BMI, FBG, HOMA-IR, IL-6, IL-1 and hs-CRP compared with the control group. The P value is <0.0001 for AST, ALT, ALP, HbA1C and for the markers of systemic inflammation. In our present investigation we observed that biochemical markers along with HbA1C and pro-inflammatory cytokines have a wide ranging role in the pathophysiology of type-2 Diabetes by monitoring both glucose homeostasis and systemic inflammation. Significant attention to lifestyle, food habits may be a boon to lower the burden of this condition.

S022

IFCC VLP Standardization and Harmonization in Laboratory Medicine

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The practice of medicine continues to be subject to intense pressures to improve performance and decrease costs. In laboratory medicine a number of pressures drive this process: (1) Globalization of laboratory practices with standardization and harmonization across national borders, (2)Global acceptance of evidence-based medicine as a foundation for best practice setting common critical clinical decision limits; (3)High investment costs and rapid development of new technologies requiring international demonstration of cost effectiveness before acceptance by stakeholders funding health care services. The profession has vigorously responded particularly through the IFCC and national professional societies establishing alliances with international standardization and regulatory agencies as well as the In Vitro Diagnostics Industry. The establishment of the Joint Committee for Traceability in Laboratory Medicine (JCTLM) is an important achievement. This movement has harnessed significant resources to markedly develop the theory of knowledge in this area and demonstrated major progress to providing comparability with many clinical laboratory assays including cholesterol, PSA, plasma protein and hemoglobin A1c. Currently the majority of clinical laboratory analytes are not suitable for assay standardization strategies. A second approach is the Global Harmonization Consortium formed to develop an infrastructure to coordinate international harmonization activities. The encouragement and support for these developments including the provision of resources remain current issues for our profession.

S023

IFCC VLP Cancer Genomics and Personalized Medicine

Paolo Fortina

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The objective of this talk is to describe some of the emerging developments, technologies and applications in the field of

cancer genomics and personalized medicine including cancer gene panel resequencing, as well as microarrays for specific clinical applications, such as MammaPrint, OncoType Dx and AMLprofilerTM in which the results are fed into an algorithm and used to assign patients to a high or low risk for tumor recurrence to diagnose breast cancer and acute myeloid leukemia, respectively. However, while new disease-based arrays are under validation, next-generation sequencing (NGS) including whole exome sequencing, whole transcriptome with short and long non-coding RNA and targeted cancer gene panel resequencing are offering unique opportunities for major advances in our understanding of cancer. This session will highlight also the latest developments in detection, isolation and molecular analysis of CTC and cfDNA in cancer using novel platforms in an effort to realize the potentials of precision medicine.

S024

IFCC VLP
Pharmacogenetics: From Bench to Bedside

Maurizio Ferrari

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Advances in understanding the molecular basis of rare and common disorders, as well as in the technology of DNA analysis, are rapidly changing the landscape of molecular genetics and genomic testing. The past decade has seen tremendous advances in our understanding of the genetic factors influencing response to a variety of drugs.There are many challenges in moving from research data to translation topractice; we discuss some of these barriers and the approaches some health systems are taking to overcomethem. Specifically, genotype can influence drug metabolism, drug transport, and a person's sensitivity to a drug. Pharmacogenetics involves applying DNA sequence data to predict drug response and to inform drug discovery and development. Diagnostic testing for "single-gene" disorders can be done by targeted analysis for specific mutations, by sequencing a specific gene to scan for mutations, or by analyzing multiple genes in which mutation may lead to a similar phenotype. The advent of massively parallel next-generation sequencing facilitates the analysis of multiple genes and now is being used to sequence the coding regions of the genome for clinical testing. New approaches to genetic and genomic testing may be applied clinically and in being aware of the principles of interpretation of test results.

S025

Towards Value Adding Our Laboratory Service

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Active interaction from the laboratory side with the clinical team with respect to diagnostic results plays a key role in the timely management of patients in any health care set-up. Clinical case based discussion plays a fundamental role in this aspect of the practice of Clinical Chemistry. Clinical case based teaching along with interpretation forms an important aspect of teaching and training, both for medical students and for scientific staff who have the responsibility to liaise with requesting doctors regarding their patient results. A clear understanding of the underlying mechanisms of laboratory findings behind the etio-pathogenesis of the clinical conditions of patients together with its clinical implications is extremely useful and contributes significantly towards providing value adding to the service. As laboratory custodians, we routinely perform and validate test results from requests that cover a wide range of clinical situations. Some diagnostic findings may require further elucidation from a diagnostic perspective as it might reflect a secondary clinical setting which needs delineation and testing to ensure that appropriate clinical management ensues. To achieve this, it is paramount that a good understanding of the laboratory findings is evident and Case Based Learning (CBL) allows acquisition and translation of such knowledge effectively to the patient's bedside. A range of diagnostic cases and results covering various aspects of hormonal and biochemical investigations will be presented during the CBL session. The successful outcome of these sessions will allow us to effectively offer laboratory resources and knowledge to assist and advice our clinical clients in a timely and cost effective manner.

S026

Evaluation and Mitigation of Lead Poisoning in Developing Countries

Thuppil Venkatesh

National Chairman, Indian National Society for Lead Awareness and Research Director National Referral Center for Lead Projects in India (NRCLPI) and Professor Emeritus Biochemistry Department SJMC Bangalore, India

Lead poisoning is a global problem and of great concern to policy makers and implementers. Decisions are taken based on the data available on blood lead level and environmental lead. Most of the countries do not have adequate date to take country level decision in the management of lead poisoning. WHO report has

regaled that most of the lead poisoning occurs in developing countries and most affected are children during their growth and development. In India prior to the introduction of unleaded gasoline in March 2000 51.6% of children below 12 years of age were found having elevated blood lead level (BLL). We do not have enough data in India to evaluate the impact of post unleaded gasoline era. Various alternate sources of lead are found and the problem continues to exist at an alarming level. Recent studies by the NRCLPI on 960 traffic policemen who are exposed to environment contaminated with lead were found to be having elevated BLL which reflects the possibilities of general population still exposed to lead from vehicular exhaust as lead free petrol is not provided. Apart from this study results from NRCLPI has indicated that soil lead continues to remain high and the major source of contamination of water. Through the life cycle lead is found getting back to human life. Other potential sources of recent days in developing country are paints with high content of lead and cosmetics apart from traditional medicine. Author has made an analysis and evaluated the economic impact of lead poisoning in a society demanding much of the corrective budgetary requirement especially in developing countries. Recommendations through the white paper are made to minimize the environmental contamination through regular monitoring of both BLL and environmental lead and to mitigate the lead contamination. In order to achieve this appropriate industrial policy and promotion board with preventive measures are suggested. NRCLPI and INSLAR are working towards this with the help and assistance from over thirty referral centres established across the country. Both world Health Organization and National organizations have come forward towards the evaluation and mitigation of lead poisoning in India.

S027

Lead in Health and Disease

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Widespread use of Lead has caused extensive environmental contamination and health problems in many parts of the world. It is a cumulative toxicant that affects multiple body systems, including the neurologic, hematologic, gastrointestinal, cardiovascular, reproductive and renal systems. Lead exposure is estimated to account for 0.6% of the global burden of disease, with the highest burden in developing regions. Childhood lead exposure is estimated to contribute to about 600,000 new cases of children with intellectual disabilities every year. The developing nervous system of children is most susceptible to deleterious effects of lead. Subtle effects on intelligence quotient (IQ) are expected from blood lead levels at least as low as 5 μg/dl and the effects gradually increase with increasing levels of lead in blood. Prenatal exposure of Lead also has been documented with developmental delays. In adults low lead exposure has been correlated with rise in systolic blood pressure as well as has been found to effect sperm count and sperm motility. Relationship of lead with anaemia is well documented in adults as well as children. Recent reductions in the use of lead in petrol, plumbing and solder have resulted in substantial reductions in blood lead levels. However, significant sources of exposure still remain, particularly in developing countries. Effect of lead on different organ systems as well as potential research areas will be discussed.

S028

Lead Poisoning in Occupational Workers and Their Families

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Lead is a poisonous metal and is widely used in daily life because of its good chemical properties. Increased use of lead, its excessive inhalation and ingestion can adversely affect major biological functions in the human body. Plumbism or Lead toxicity is defined as a toxic condition caused by the ingestion or inhalation of lead. In 1991, the Centre for Disease Control and Prevention of the United States Department of Health and Human Services recommended that lead toxicity occurs when blood lead levels are equal to or greater than 10 μg/dl. Since then these values have been revised many times by various agencies including the World Health Organization and there are certain reports that even levels as low as 2 μg/dl may cause toxicity. Sources of lead exposure and toxicity include old piping, and working in certain occupations such as battery recycling workshops, printing, plumbing, painting, the destruction of old houses etc. It is very important to identify the probable sources of lead exposure, especially those that may be of significance to children and infants. Lead based paints are the supreme source of the lead poisoning in the developing world. The rate of lead absorption is affected by age, sex and food intake. Moreover, there is higher rate of absorption after fasting than when lead is ingested with a meal. Furthermore, lead absorption is increased when there is decreased intake of essential metals such as iron, zinc and calcium as well as poor nutritional status. All these things are to be kept in mind while devising ways and means to prevent lead toxicity.During last couple of years we have carried out a number of studies in painters, battery workers and their family members. We not only observed high blood lead levels but also found perturbed biochemical profiles in most of the subjects. Detailed findings and observations will be discussed during the meeting.

S029

A Decade of Lead's (Pb) Journey in India from Lab Top to Beneficiaries/Masses

N Shashidhara

Principal Advisor NRCLPI, National Secretary INslar

"If your - Petrol to be unleaded, paints to be lead safe, water to be lead free -What about your CHILD? We are familiar with the naturally available heavy metal - Lead (Pb). This metal has been used for centuries by man because of its useful properties and it is found that the Romans were using this metal lead for making vessels to store wine in it. Interestingly in 1713, the hazards of lead were first reported by Bernardo Ramazzini in potters who used lead for glazing. During the 18th century, Benjamin Franklin noted the toxic effects of lead on printers, plumbers and painters who used lead in their trades. This shows that the scientific community was/ is working hard in experimenting and recording the ill effects of lead poisoning on living beings and hundreds of papers are being published on lead related subject every year. But we find that there is wide a gap in transmitting the information of ill effects of lead on human beings – that is From the Labs of the Scientists to an ordinary man in the society. For want of this information / awareness about the dangers of Lead poisoning – now we can see that every individual in the society is being susceptible directly or indirectly to the dangers of lead poisoning - specially the children and women who are so vital to the growth of the society. Today the abundant supply of lead and for its useful properties like softness, malleability and easy to handle and in economic terms its cost effectiveness qualities made the lead a favoured – wonder metal/ material by the technocrats. This wonder metal is being used freely in making many of the products like – Paints, Batteries, Solder, Pottery Glaze, Water and Sewer Piping, stained glass, crystal vessels, ammunition, jewellery, and toys and in some of the cosmetics and traditional medicines etc. In short the lead shaping our society into a LEAD SHAPED SOCIETY. And Lead got the dubious distinction of becoming No. 1 environment pollutant. The lead toxicity or lead poisoning remains a matter of public health concern, and the awareness about its toxic effect has gained a lot of importance over the recent years. There has been a growing interest in monitoring heavy metal contamination in the developed countries like the USA, European countries, Germany etc and the Government has put lot of restrictions on industries using lead and its proper disposal. In the USA as of 1978, the Consumer Product Safety Commission (CPSC) banned the use of lead in residential paint. As a result of this ban, buildings built after 1978 are not considered to be a risk for lead contamination from paint. But in a developing country like India there is no restriction with regard to the use of lead in paints and least concern to the dangers of lead poisoning to the consumers. In the Indian context prior to 1990 the Lead related health problems were rarely discussed – the project lead free of the George foundation drew the attention of the concerned both in Government sectors and also among the NGO's the dangers of lead poisoning and the urgent need to create awareness. The St John's Medical College of Bangalore took the initiative in establishing - The National Referral Centre for Lead Poisoning (NRCLPI) now it is the National Referral Centre Lead Projects in India a centre totally dedicated address the lead related issues – over the years it has designed and developed many interesting programmes to create Lead Awareness among the general public and others who are working in the lead related areas to mention a few are – In order to create awareness about lead in the society NRCLPI with QCI has initiated various programmes in different parts of the country. The main focus is to bring home the message of lead - uses and abuses of lead and lead related hazards and preventive measures etc - (1) LEADer(ES, HS) - LEAD Educator (Educational sector,Health sector, craft sector, NGO) This awareness programme is designed for creating Lead educators in all educational institutions by the competent members of NRCLPI. This programme enables the LEADers to conduct the programmes independently in their respective institution on Lead Awareness. (2) SMILE (Seminar on Methods & Implementation of Lead Education) This lead awareness programme is designed exclusively for Pre-School Educators, parents & aganwadi workers in mind as the children spend most of their active time with them in learning, playing using the play equipments, toys etc. (3) STEP (Student Talent Exploration Projects) – NRCLPI is sponsoring short term lead related research projects from the city colleges of different states. Initially it was started in Bangalore where 50 Colleges had come up with lead analysis in different samples. These STEP scientists will be engaged in making Lead Maps of Bangalore, Hyderabad and Chennai. The lead map will work as a guideline for people and policy makers and it increase awareness about our exposure to lead pollution in a particular place. Data is collected from all possible sources; soil, air, water, paints, ceramic and plastic articles, and blood. (4) Eco – Ganesha and Durga. It was found during some of the festivals lot of lead containing paints are used to make the idols and pendals resulting in lead pollution in water and air. The NRCLPI in association with Craft Councils, State Pollution Control Boards initiated lead safe festivals. (5) Workshop for persons engaged in Lead related Industries:- This programme is meant for workers working in battery, paint, automobile industries etc. (6) LEAD wise clubs- LEAD ('Y's) clubs. In order to create an awareness about lead among students, societal groups, eco-clubs,etc it is proposed to establish LEAD ('Y's) CLUBS in various sectors. These clubs conducts programmes and activities related to Environment and more focused in the area related to lead. (7) QPEC:- (Quality Playing Environment for children) This is a new initiative taken by NRCLPI which addresses the organisers of Balbhavans, children's parks and schools in the country to go for lead safe paints for their wall & playing items so that children can play in a lead safe environment. LEAD Clinics- The NRCLPI has set up several lead clinics to conduct Blood Lead Level (BLL) tests this is in order to help and guide the persons suffering from Lead poisoning now more than 25 Lead clinics are functioning all over the country. The Indian Society for Lead Awareness and Research (INslar) an

all India body is playing an important role in creating – Awareness about lead – its membership is open to all interested.

S030

A Case Based Approach to Biochemistry

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Case Study based method is a teaching learning methodology, which fosters exposure of the medical students to the patients. It forms a crucial part of the initiation process into medicine and gives an opportunity to bring social relevance to basic science learning. Cased based learning will serve to be motivational to learners and it can provide scenarios from which complex concepts can be more easily understood. Clinical intervention in the early years of the medical college shall help to improve not only exposure to clinical skills but also help the students to see Biochemistry in a new light of patient care. The importance of Biochemistry in diagnosis / prognosis of diseases will also be appreciated by the students. At the institutional level the positive gains will be: 1. Exposure to faculty to work on cases; 2. Exposure of students to clinical application of the theoretical aspects of Biochemistry; 3. Instilling into the students the relevance of social accountability; 4. Active and self-directed learning in students will increase and improve. It will also enhance teacher – student and teacher – teacher interactions. The experience of our department shall be discussed. The results of which suggest that students felt Case Based Study provide a greater satisfaction compared to didactic lectures. The students also felt that it improved their reasoning skills, communication skills, helped them to understand the etiology better and most of all made a complicated concept easier to understand. It also brought out the interconnections between various metabolisms and basic concepts of clinical biochemistry were better understood and grasped. Thus, case based approach is an important avenue for introduction of Early Clinical Exposure in Biochemistry and instilling social accountability.

S031

Integrating Biochemistry in Medical Curriculum-Gulf Medical University Experience

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A competency based integrated curriculum was introduced in September 2008 at Gulf Medical University, UAE.This was done keeping in view the changing trends in medical education worldwide and the learning needs of the graduating doctor. The curriculum contains the essentials of all six components of the SPICES model and builds on the molecular concepts of life.The 5-year-curriculum is completed in three phases. Phase I (Year 1) comprises of seven courses dealing with the foundations in Basic Medical Sciences. Phase II (Year 2&3) covers eleven organ systems and Phase III (Year 4&5) covers the clinical clerkships. The concepts in Biochemistry have been integrated with the structure-Function relationships of biomolecules and etiopathogenesis of different organ systems. The learning of Biochemistry has been made relevant for the graduating doctor by stressing on the molecular basis of health and disease. Horizontal and vertical integration of curriculum and frequent visits to basic and clinical sciences has proved the role of Biochemistry in molecular and clinical diagnosis of diseases and their management.The salient features of successfully implemented curriculum will be shared and the participants will be guided in integrating the teaching of Biochemistry in a traditional curriculum.

S032

Integrated Assessment for Biochemistry in Medical Curriculum-The Gulf Medical University Experience

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A competency based integrated curriculum was introduced at Gulf Medical University, UAE in September 2008 and has been successfully run for six batches of medical students. The curriculum is delivered in 3 phases over a period of five years. The strong points about the curriculum are its organization and the delivery of the content and the remarkable achievement of the graduating students in various international competitive examinations. An intense continuous assessment process is the hallmark of the curriculum. The internal assessment of different components of learning comprises of 60% marks and each of the three end of the phase examinations carry only 40% marks. Since "assessment drives learning", an integrated assessment is very important for learning in an integrated curriculum. The learning of Biochemistry during the foundation year and in the organ systems and clerkship courses is assessed in an integrated way using written (Multiple Choice Questions, Modified Essay Questions, Extended Matching Items, Short Answer Questions), practical (OSPE)and clinical (OSCE) methods. The participants will be given examples of integrated assessment in Biochemistry and task based hands on interactive session will be conducted to plan integrated assessment for the courses taught by them.

S033

IFCC TFYS
The Future Specialist in Lab Medicine: Realistic Expectations and Curricular Needs in Research

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Science and technology are crucial in modern medicine; societies devote enormous amounts of time, money and effort to developing new diagnostic and therapeutic procedures. Laboratory Medicine education and medical practice have been changed markedly by the success of biomedical research in recent decades and by the increasing role of Health authorities in medicine. The future specialist in Lab Medicine will require a strong background in the basic medical sciences as well as highly developed clinical skills. He will need the capacity to reason scientifically and rigorously in relation to technologies as well as clinical issues. Medicine should be viewed as an integrative science. The development of a robust Lab Med and clinical research is needed to improve health care and wellness of the population. It is important to consider how to influence biomedical and clinical research funding priorities, promote mechanisms to train specialists in Lab medicine and other health care professionals to conduct biomedical and clinical research and how to encourage health care providers to follow evidence based medical practice. Consensus can emerge on multiple issues, including international collaboration, the need for a core biomedical and clinical research curriculum for training the new cadre of clinical researchers, joint advocacy for increased funding of biomedical and clinical research and for the education of policymakers and the public on the benefits of biomedical and clinical research. Specific recommendations can be made on mechanisms for recruitment, training, and retention of biomedical and clinical research trainees and mentors. Steps should be clarified to overcome career disincentives and to encourage use of web-based and continuing-medical-education-based mechanisms to bring specialists in Lab Medicine up-to-date on issues in and results of biomedical and clinical research.

S034

IFCC TFYS
Introduction to Research Design

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Research refers to a search for knowledge and is an art of scientific investigation. One can also define research as a scientific and systematic search for pertinent information on a specific topic. Research comprises of defining and redefining problems, formulating hypothesis or suggested solutions; collecting, organizing and evaluating data; making deductions and reaching conclusions; and at last carefully testing the conclusions to determine whether they fit the formulating hypothesis.The main aim of research is to find out the truth which is hidden and which has not been discovered as yet. The purpose of research is to discover answers to questions through the application of scientific procedures. This is to gain familiarity with a phenomenon or to achieve new insights into it or to test a hypothesis of a causal relationship between variables. There are various descriptions of the types of research which brings to light the fact that there are two basic approaches to research, viz., quantitative approach and the qualitative approach. Research is a function of researcher's insights and impressions. Such an approach to research generates results either in non-quantitative form or in the form whichare not subjected to rigorous quantitative analysis. The study of research methodology gives student the necessary training in gathering material and arranging, indexing them, and also training intechniques for the collection of data appropriate to particular problems, use of statistics, questionnaires and controlled experimentation and in recording evidence, sorting it out and interpreting it. Research process consists of series of actions or steps necessary to effectively carry out research and the desired sequencing of these steps. Thus, knowledge of how to do research will enhance the ability to evaluate and formulate the research design.

S035

IFCC TFYS
Literature Review using the Internet Support & Theoretical Approaches - Taking Right Step Forward

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*"Research is formalized curiosity; it is poking and prying with a purpose" ….**Zora Neale Hurston.*** Research is an important essential activity that contributes to ongoing development of science. Special areas like Science and Medicine are continuously progressing which makes it even more important to keep pace with such development. To an aspiring professional, research bestows a sense of satisfaction and achievement. Research activity is increasingly undertaken by those doing post graduate and graduate studies. There is no bar or boundary that is stipulated as to when research should begin. One of the most difficult steps when considering research is to choose a right topic of interest. This usually begins as an idea that needs to be explored, a view that needs to be stated, or a hypothesis that requires proof and relevance. Research to achieve this involves several steps which have to be systematically approached so that the desired outcomes (whether positive or negative) can be achieved. This job of locating the background information through systematic literature review nowadays has been made easier through web based "virtual library" facilities. These resources were not easily available in formative years when a career in research was contemplated. Appropriate search and review of literature dictates, directs and leads to better beginning and conduct of research. Formulation of methods and a planned approach should be followed for ensuring that suitable steps are taken for the proposed work so as to provide answers for the research questions raised. This is dependent on the literature review conducted by the researcher. Methodology, therefore, is crucial as this will help delineate the experimental studies and outcomes. Interactive approach to discussion and networking with peers and fellow researchers play a key role in the successful conduct of research. Experimental approaches and analysis defines the information that is obtained. Other aspects of research work that needs attention include sampling, randomization where needed, presentation of the experimental findings including statistical analysis of observations and ethical and legal considerations for conduct of the study. Discussion and Conclusions have to be soundly derived, based on the experimental findings. An abstract of the research findings has to be prepared that summarizes the salient features of the findings

S036

IFCC TFYS
Designing Using Quantitative and Qualitative Method

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A research question is an uncertainty about something the investigator wants to resolve. This research question should be feasible, interesting, novel, ethical and relevant. Sequence of any research involves definition of problem, specifying objective, selecting design or type of study, selection of study population, collection of data, analysing data and determining conclusion.The philosophical approach to research is basically of two types: empirical and theoretical. Health research mainly follows the empirical approach, i.e. it is based upon observation and experience more than upon theory and abstraction.Empirical research in the health sciences can be qualitative or quantitative in nature. Qualitative methods are largely unfamiliar to health professionals and researchers. Quantitative methods include measurement and analysis of causal relationship between different variables whereas a qualitative method of study does not seek to quantify or enumerate, it does not measure. Though it does not measure, a qualitative data can be measured quantitatively. Here the measurement can be done in taxonomy or classification. A qualitative study design may not specifically test any hypothesis, rather it generates hypothesis to be tested by quantitative study design. Generally, health science research deals with information of a quantitative nature. It involves identification of the population of interest, the characteristics (variables) of the individuals in the population, and the study of the variability of these characteristics among the individuals in the population. The epidemiological approach is based upon statistical principles in the structuring of research design. In this approach, research can be divided into that which is basically observational in type, and that which is experimental.Testing of hypotheses is best done by experimental studies, where all the factors other than those under consideration can be controlled and it is intended to establish causality between variables. Experimental study designs include clinical trial and community trials. However, in human diseases, this is not often possible, due to ethical and practical considerations. This can be achieved by carefully designed observational studies. These analytical observational studies can be retrospective (case-control) or prospective (cohort and retrospective cohort studies). Here, observations on cause and effect differ by way of a period of time and inference of associations can be made. Observational studies also include surveys of the cross sectional type which are considered to be hypothesis-generating studies. Observational studies differ from experimental studies in that there is no direct intervention by the investigator. In either approach, statistical reasoning using the

laws of probability guides the inferential process. Some basic assumptions are made about the population, its characteristics and the probability distribution, and the likelihood of the observations supporting or contradicting the stated hypothesis, is evaluated. Based on these calculated probabilities, the hypothesis is accepted or rejected.

S037

IFCC TFYS Drafting a Research Proposal

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The way in which a research proposal is structured and worded will have a significant impact on its likely acceptance. The following guidelines are recommended: 1) Allow plenty of time to prepare the submission; 2) Choose a research funding organisation that has a track record of funding your type of proposal and/or your area of expertise; 3) Formulate your research proposal into a hypothesis or series of questions, which will be investigated by the proposed research. This preparation will inform the 'Aims, Objectives and Outcomes' of your proposal; 4) Plan the experiments/investigations you propose in detail. A flowchart may be helpful. For each step estimate the likely time interval and the resources (patients/equipment/consumables etc.) that you will require. This will help you to complete the Methodology and Budget sections of your proposal; 5) when preparing the draft proposal follow exactly the rules/guidelines recommended by the research funding organization; 6) Use simple language and keep the proposal concise and positive. Reference key areas but don't include large numbers of references. Think who may act as a referee when selecting your key references; 7) be realistic about time scales, resources and likely outcomes; 8) Share your draft proposal with collaborators, senior colleagues and your family to get different levels of feedback. If possible include relevant patients in this consultation; 9) Finalize your application in the light of feedback and include all necessary approvals (ethical, institutional, collaborators etc.); 10) Submit the complete application ahead of the deadline together with a simple covering letter.

S038

Saliva Metabolomics: A Potential Tool for Detecting Systemic Diseases

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Saliva is a non-invasively available and informative biofluid which enables us frequent medical experiments, which potentially increase the chance of the early diagnosis of various diseases. We have developed metabolomics technologies that can simultaneously identify and quantify hundreds of low-weight-molecules, named metabolites, in any cellular systems. We established measurement protocols and developed data analysis systems for the use of capillary electrophoresis-mass spectrometry (CE-MS). Here, we introduce the recent technologies named non-targeted metabolomics which detect all possible metabolites without any pre-definition of analytical targets and therefore suits the studies of biomarker discoveries. We also reports the salivary metabolomics studies to find out potential biomarkers to diagnose various types of cancers as well as its validation studies.

S039

Genotoxic and Mutagenic Activity of Bisphenol A an Endocrine Disruptor and Understanding its Mechanism of Action

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There is an increasing concern about the exposure to environmental estrogenic chemicals in a number of human health disorders viz. testicular cancer, precocious puberty, low sperm count, hypospadias, and cryptorchidism. One such estrogenic chemical is Bisphenol-A (BPA), a synthetic monomer used in production of polycarbonate plastics, epoxy resins, food packaging, dental sealant and lacquers for food cans. Human beings are exposed to BPA, as it leaches from the inner lining of tin cans and microwave containers during heating into the food materials. Recent studies in humans depicted its association with recurrent miscarriages and male infertility due to sperm DNA damage indicating that BPA might have genotoxic activity. The aim of the present study was to assess the possible genotoxic effects of BPA exposure by using a battery of genotoxicity assays in somatic and male germ cells using rat model. The data obtained in the present study demonstrated that BPA is a genotoxic compound and might be a mutagen to male germ cells, and may impair fertility. The levels of various

antioxidants enzymes were estimated to decipher the genotoxic mechanism of BPA, which suggest that oxidative stress, could be one of the possible mechanisms for genotoxic activity of BPA.

S040

Nuclear Receptor Peroxisome Proliferator–Activated Receptor: Potential Transcriptional Nodal Point of Fertility Gene Regulation by Endocrine Disruptors

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PPARs are metabolic nuclear receptors and, in general, mediate their effect through their ligands (Endogenous ligands- PUFA and Exogenous ligands- Herbicide, Thiazolidinediones, plasticizers) and alter target gene expression. Recently, we have cloned, characterized and identified a novel ovary-specific PPARγ transcript. And, elucidated molecular mechanism how PPARγ, could influence the ovarian function using CLA and rosiglitazone has been chosen as endogenous and exogenous ligands, respectively. Result showed that both, CLA and rosiglitazone, inhibits FSH and IGF1 induced granulosa cell proliferation, aromatase, *GATA4, IGF1* mRNA and estradiol-17β production. Western blot analysis of total cell lysates revealed that CLA and rosiglitazone intervene the IGF1 signaling by up regulating the PTEN expression and hence decreasing the pAkt. In addition, acetylated histone expression was found to be down regulated in cells treated with PPARγ ligands. In conclusion, result of present study showed that PPARγ transcript could be a potential gateway for endocrine disruptors and could affect key gene encoding rate-limiting enzymes of estrogen biosynthesis. (The work is supported by DBT, DST-DFG, DST-DAAD and ICAR).

S041

A Study on Association of Obesity with Male Reproductive Hormones in South Indian Young Adults

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Obesity has become a pandemic and obesity during childhood or young adulthood usually persists to adulthood and is a common link to Diabetes mellitus, CVD, hypertension, metabolic syndromes and also gives rise to reproductive disorders such as PCOS in females, infertility, and sexual dysfunction in both genders. In obese males high BMI and WHR (waist-to-hip ratio) is associated with low testosterone and GH levels and high estradiol, SHBG, cortisol and hyperinsulinaemia which may lead to infertility or sterility in males. To study the association of obesity with altered levels of reproductive hormones like serum estradiol, testosterone, thyroid hormones, SHBG, DHEA in young adult obese males of South Indian origin. The study was done with 32 obese and 21 non-obese young adults in the age group of 21-35 years. The routine parameters and lipid profile were assayed in semiautoanalyzer by standard kit methods and thyroid profile, SHBG and sex hormones by standard ELISA methods. BMI, WHR, WC were significantly high in the obese group ($P<0.001$). TG and sdLDL-C (small LDL-C) was significantly raised in the obese group. Serum testosterone was significantly low ($P<0.01$) whereas no significant change of SHBG and DHEA was there for the obese group. There was hyperinsulinaemia ($P<0.05$) and high estradiol in the obese group. BMI and WHR correlated significantly negatively with testosterone and positively with estradiol level ($P<0.001$). Obesity is preventable and hence by modifying diet and lifestyle in young adults, reproductive hormone disorders may be obviated.

S042

Angiotensin Converting Enzyme Gene Insertion Deletion (I/D) Polymorphism and its Association with Urinary 8-OH dG in Essential Hypertension

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Hypertension is a risk factor of Cardio-vascular dysfunctions. The Rennin- aldosterone system plays a significant role in the generation of reactive oxygen species (ROS) in essential hypertension. Angiotensin converting enzyme (ACE) gene I/D polymorphism is associated increased levels of ACE. 8-OH deoxy guanosine is an end product of oxidative damage of DNA invio.It is widely thought that continuous damage to DNA is a significant contributor to various diseases like hypertension, atherosclerosis, cancer etc.The aim of the present study is to know the distribution of ACE (I/D) polymorphism and its relationship with 8-OH dG in essential hypertensive patients. 208 clinically diagnosed essential hypertensive patients and 220 control subjects included in this study. Genomic DNA extracted from whole blood and PCR was performed for analyzing Insertion (I) and Deletion (D) polymorphism. ACE genotypes are D/D, I/I and I/D . Spot urine samples used, for the analysis of 8-OH dG levels by using ELISA method. The distribution of D/D genotype of ACE gene was significantly higher

(38.9%) in patients when compared to control subjects (20%) odds ratio 2.5 (P<0.001). Urinary 8-OH dG levels are significantly increased in hypertensive patients when compared to controls. D/D genotype is strongly associated with elevated levels of 8-OH dG when compared to I/D and I/I genotypes.The study demonstrates deletion (D/D) genotype of the ACE gene might be an risk factor for the generation of ROS which leads to oxidative damage to DNA in hypertensive patients.

S043

Stress in Healthcare; Givers & Takers

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Occupational stress has been long standing concern in the healthcare industry. A survey conducted in USA, shows that 1 in 2 physicians has symptoms of burnout. It also came out that it was not related to personal attributes, but were due to deep rooted issues in the system of care delivery. It has been established that staff get deeply involved with the ailing patients and have frequent exposure to death/dying. This high grief exposure leads to higher stress among the staff. Healthcare industry is highly human intensive and there is increasing demand on staff to equip in multiskilling. Emotional demands of dealing with sick patients, coupled with excessive work load are resulting in stress among all categories of hospital staff. Management is generally focused on taking care of patients and forget to realize that their own staff were under stress and that would affect the patient care. It is therefore important that management must implement strategies to reduce burnout while also promoting productivity or patient care. In case of patients, stress comes more naturally. There is fear of un-known. They are away from their home, near & dear. They are confined in wards and are in contact with other patients. They have to take food which at times is not to their taste. All these factors are source of stress. What is more important that stress caused by hospitalization may hinder patient recovery? It can even cause life-threatening psychological changes. It is therefore necessary that staff is aware of such conditions and are trained to have continuous communication with patients with positive outcomes. These can even help staff to cope up their own stress. Hospitals therefore need to evolve strategies which deal with stress of their staff, patients and even integrated ones so as to be self-sustaining.

S044

The Consequences of Antarctic Conditions and Ship Voyage: Various Body Responses in Indian Expedition Team

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Antarctica - remote, isolated and frozen all year, is the most untouched and undisturbed region on the planet. Science in Antarctica offers many advantages over anywhere else on earth. Thus, Antarctic milieu comes with extreme cold, intense UV radiation, white-outs, varying magnetic fields, sleep deprivation, altered circadian biorhythms, memory loss, isolation, - causing significant physiological and psychological stresses. Many aspects of Antarctic life have become the topic of medical research over recent years and few articles have been published on the lifestyle of Antarctic expeditioners. We carried out investigations to observe the effect of multiple stresses like ship borne journey, isolation, cold, Magnetic field and UV exposure, on Immunological, Biochemical and Proteomic parameters, on two participating Indian teams of 28th Indian Scientific Antarctic Expedition, from December 2008 to December 2009. The study conducted on expeditioners indicated that ship borne journey and acute exposure (summer team) to Antarctica increased Th1 cytokines, while both acute and chronic exposure (wintering team), to Antarctica persistently up regulated the sIgA level. Therefore, sIg-A could be considered as possible biomarker for stress under extreme environmental conditions. Hsp-60 was also found to be increased throughout the expedition in both the teams. Activation of complement system during the ship journey in summer team members as well as during the month of August in the winter team was also observed. Further, analysing NMR data it was observed that there were significant variations in concentrations of number of metabolites which clearly suggests that there were alternations in different metabolic pathways, both during the ship journey as well as wintering in Antarctica. No specific patterns of disease have, as yet, been predicted by these various parameters; however, these findings are important because there might be long-term health implications due to significant alterations in various body components.

S045

Stress: Onset and Progression of Diseases

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The term "stress" was first used by the endocrinologist Hans Selye in the 1930s to identify physiological responses. Stress, defined as a state of threatened homeostasis, mobilizes a complex spectrum of adaptive physiologic and behavioral responses that aim to re-establish the challenged body homeostasis. The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) constitute the main effect or pathways of the stress system, mediating its adaptive functions. Stress affects most of the people in some way or the other. Stress responses could prove beneficial if they are within the threshold limit of an individual. However, repeated stressful situations beyond threshold limit put a strain on the body that may contribute to physical and psychological problems. Stress causes several diseases; largely because of its effects on immune system, oxidative stress and inflammation. Acute (sudden or short-term) stress leads to rapid changes throughout the body. During stress almost all body systems (heart and blood vessels, immune system, lungs, digestive system, sensory organs, and brain) are affected. Chronic (long-term) stress can have real health consequences and should be addressed like any other health concern. Fortunately, research is showing that lifestyle changes and stress-reduction techniques including herbal therapy can help people to manage stress. The presentation will focus on stress and its role in progression of diseases.

S046

Nutritional Stress, Calorie Restriction and Voluntary Food Denial

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Adequate nutrition plays vital role in health, malnutrition as well as over nutrition is harmful and lead to metabolic disorders. Same time intake of food itself causes postprandial oxidative stress. Calorie restriction up to certain extent (about 40% low intake than usual) is beneficial as it reduces oxidative stress and has been found to increase life span of organisms. Calorie restriction is achieved by voluntary food denial or deprivation and is practiced by many people either for health benefits or due to religious practices. A study was conducted on 34 participants (11 female, 21 male) who consumed only boiled water for 7days. A classical starvation response was noted with decrease in body weight mainly due to dehydration, mobilization of fatty acids, disturbances in electrolytes, increased acetone levels in blood. Subjective feeling of discomfort was more during initial three days and improvement was noted with progression of fast. After 7 days volunteers resumed their food intake with excessive intake of sugars which caused significant increase in plasma triglyceride levels. In another study with deferent degree of calorie restriction on 50 volunteers (20 to 40%) beneficial effects like reduction in body fat and oxidative stress were observed. Only drawback of these interventions in control of bodyweight is feeling of hunger and quick gain of fat mass once intervention is failed due to poor adherence.

S047

Heat Stress in Rajasthan

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Tropical latitudes substantiate the warm climatic conditions. They range from arid and semi-arid terrain of desert to the hot humid coastal regions. Rajasthan desert pose challenge for inhabitants of the area and more to them who carry out operations (physically strenuous activities) under such conditions. Soaring ambient temperature along with intense solar radiation, warm winds and sandy terrain aggravates the hardship of these areas. Air temperature alone does not provide an adequate measure of thermal load. The severity of a given environment depends also on factors such as radiant temperature, humidity and the amount of air movement. Human beings are homoeothermic creatures whose physiology attempts to maintain a constant core body temperature of 37°C (range 36 to 38°C). Obviously, this requires balancing of the body heat production with heat loss. Environmental stress disturbs this homeostasis and lowers the physical fitness and performance of man. Heat stress is one of the effects of elevated ambient temperature. It can lead to heat maladies ranging from as mild as headache and prickly heat to as severe as hyperpyrexia and heat stroke. Heat illness can further broadly be classified into exertional heat illness and non-exertional heat illness. Physical activity under extreme hot environment leads to maladies which are classified under exertional heat illness. They vary from heat cramps to heat stroke. Non-exertional heat illnesses envelop maladies like heat rashes, heat syncope and dehydration. DIPAS being a DRDO laboratory is into research in the field of environmental physiology with the mandate to help alleviate such incidents among our fighting forces. Studies have lead to development of replenishment drink – DIP-SIP to tackle the detrimental effects of dehydration to some extent. Exploration in the field of auxiliary cooling devices to keep soldiers body cool has lead to development of man mounted cooling system

(MMACS). Wearing these portable apparatus would help the forces maintain their body temperature within permissible physiological limits while working under high ambient heat. Among volunteers consuming DIP-SIP depletion of sodium was less compared to control batch (from 141meq to 139meq in control groups and from 141meq to 140.8meq in experimental group). Apart from electrolytes core temperature, skin temperature and heart rate were also maintained while consuming the formulation (control group had core temperature 37.4^{o}C while after consuming DIP-SIP temperature was maintained at 36.8^{o}C at end of exposure at 45^{o}C 35%RH). Research on auxiliary cooling has shown that participants wearing MMACS had difference of 0.26^{o}C between their initial and final core temperature. Fluid consumed by participants using MMACS was 331ml / hour as compared to 365 ml/hour in control group. These help tackle the problems of heat dehydration and their related ailments.Work carried out at DIPAS in varied magnitude has helped us better understand the effects of harsh environment on human body. The relentless efforts have been reflected in form of solutions to relief and ameliorate the deleterious effects of harsh temperatures on human body.

S048

Regeneration of Infarcted Myocardium by Genetically Modified Stem Cells

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Implantation of mesenchymal stem cells (MSC) is becoming an exciting new method forpromoting repair of infarcted myocardium. In this study, we use an adenoviral vector encoding Thioredoxin-1 (Ad.Trx1) to genetically modify MSCs prior to implantation. Trx-1 has been established as a redox regulator of growth and transcription-factors as well as a cofactor. It has also been shown to be a potential antioxidant. We wanted to study whether Trx-1 engineered MSCs are capable of improving cardiac function and angiogenesis in a rat myocardial infarction (MI) model. In order to do so, rat MSCs were cultured and divided into three groups: MSC, MSCLacZ and MSC-Trx1. The cells were assayed for survivability, proliferation, and differentiation potential. Additionally, rats were randomized into control Sham (CS), control MI (CMI), MSCLacZ MI (MLZMI) and MSC-Trx1 MI (MTRXMI) groups (n = 20 per group). MI was induced by permanent occlusion of the LAD immediately after which MSCs preconditioned with either Ad.LacZ or Ad.Trx1 were administered at 4 peri-infarct areas. We observed increased proliferation of MSC-Trx1 cells *in vitro* that maintained pleuripotency to divide into cardiomyocytes, smooth muscle and endothelial cells. In treated rats, capillary density increased in the MTRXMI group when compared to the both the CMI and MLZMI groups. Western blot analysis showed increased expression of VEGF, HO-1 and CXCR4 and decreased expression of TXNIP in the MTRXMI group. Increased intercellularconnections, measured by Cx-43 expression, were seen in the treatment group. Echocardiography showed improved ejection fractions and fractional shortening in Trx-1 treated mice when compared to LacZ and control mice. Additionally, picro-sirus red staining showed Trx-1 treated mice had decreased levels of fibrosis in the myocardium. Trx-1 pretreated MSCs provide protection against myocardial injury via induction of VEGF expression, promotion of neovascularization, reducing fibrosis and increasing functional recovery. Ischemic damage cause by decreased blood flow may be reversed in various cardiovascular diseases by treatment with MSCs preconditioned with Trx-1. The long term clinical management of illnesses, such as MI or peripheral vascular disease, may benefit from theangiogenic properties of Trx-1.

S049

A Bis-resorcinol Congener of Resveratrol as an Anti-ulcer Compound

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The dietary hydroxystilbene, resveratrol (Resv) is credited with several beneficial effects such as cardiovascular and neuro-protection and even cancer chemoprevention. Regardless of these, it also shows different contraindicative properties, especially to the gastrointestinal (GI) tract.The aim of the study was to formulate a resveratrol congener as a new chemopreventve agent without GI toxicity and rationalize its mode of action. Swiss albino mice were ulcerated with indomethacin (IND) followed by treatment with the test samples, and various biochemical and signaling parameters of the IND and treatment groups were assayed. Suitable inhibitors were also used to establish the biochemical mechanism. The congener was also tested for its anti-proliferative property against a host of human cancer cells. The new hydroxystilbene (HST-1) reversed the adverse effects of IND on several inflammatory (MPO, cytokines), ulcer-healing (cyclooxygenases) as well as signaling parameters in mice. Importantly, HST-1 down-regulated TNF-α and the TNF-α-mediated activation of NF-κB and JNK MAPKs. The effect of HST-1 was significantly better than that of Resv, misoprostol and omeprazole. The effect of Resv on pP38 and p-JNK was much less, while it reduced the pro-survival ERK1/2 activation. HST-1 also showed better growth inhibitions of several human cancer cell lines than Resv. HST-1 may be a potent antiulcer and anti-cancer agent. Apparently, its ability to control TNF-α induction and subsequent activation of NF-κB and JNK prevented

stomach ulceration and enabled faster healing. Moreover, the structural modification also enhanced its anti-cancer property.

S050

Chronomics of Circulating Plasma Lipid Peroxides, Antioxidant Enzymes and Other Related Molecules with Advancing Age

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The chronome (from chronos, time; and nomos, rule; time structure) of lipid components, including lipid peroxides and antioxidant defense mechanism may change with advancing age and may prove to be of physiological significance in understanding the role of oxidants and antioxidants in healthy populations of different ages. The circadian time structure of lipid peroxidation and antioxidant defense mechanisms may also relate to the aging process.The circadian periodicity of circulating plasma lipid components (Total Cholesterol, HDL- Cholesterol, Phospholipids, Total Lipids), plasma lipid peroxides in terms of malondialdehyde (MDA), antioxidant enzymes as Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione Reductase (GR) and other biochemical variables as Ascorbic acid, Uric acid, Total Serum Protein and Serum Albumin were studied under near tropical conditions in 162 healthy volunteers (103 men and 59 women; age: 07 to 75 years) with a diurnal activity from 06:30 to about 22:00 and nocturnal rest. These volunteers were divided into 4 groups, 07-20, 21-40, 41-60 and 61-75 years of age, comprising 27, 42, 20 and 14 male and 15, 18, 15 and 11 female participants, respectively. Blood samples were collected every 6 hours for 24 hours under standardized conditions. All studied variables were quantified and enzyme activities were measured with spectrophotometric procedures. A marked circadian variation was detected in all studied variables in healthy Indians of different age groups by population-mean cosinor analysis (almost invariably $P<0.001$). Changes as a function of age were observed in the MESOR, circadian amplitude and/or acrophase of many of the variables examined. A tendency for the amplitude to decrease and the acrophase to advance with increasing age cannot be considered without a scrutiny of infradian changes with periods ranging from weeks and months to decades.Mapping the broader time structure of different physiological variables with advancing age, including focus on multi-frequency components of oxidants and antioxidants will be of great importance in understanding mechanisms of aging.

S051

Kinetic and Biochemical Method for Evaluation of the Antioxidant Activity of Herbal Extracts and their Active Principles

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Oxidation of biological molecules has been postulated to induce a variety of pathological events and ageing. It has been proven beyond doubt that these damaging events are caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS). Therefore the concept of pharmacological supplementation of a defense against ROS/RNS with antioxidants has become an intensive area of research. The first step towards the development of future therapeutic agents is the comprehensive understanding of the efficacy and mechanism for the reaction of these biologically important radicals with natural and/or synthetic antioxidants. The kinetic parameters e.g., formation and decay rate constants predict the efficacy of an antioxidant and its fate after reaction. These parameters also dictate the ease with which competing reactions would occur in a bio-environment. Physico-chemical and biochemical methods in the evaluation of antioxidant activity of some important medicinal plants commonly used in India and the subcontinent would be the focus of this presentation.The systems chosen for discussion, are herbal extracts as such,curcumin from turmeric, methoxy phenols from Indian spices, dehydrogingerdione from ginger and bakuchiol from *Psoraleacoryli folia*, and some other molecules. All these examples would illustrate the potential of the pulse radiolysis coupled with kinetic spectroscopy and other physicochemical/biochemical techniques for the study of antioxidants either in the form of mixture as in herbal extract or as an isolated compound. Because of the multi-constituent mixture, it is not possible to determine the absolute rate constants for the reactions of extracts with free radicals. However, it is possible to estimate their relative reactivity in comparison with a standard, which can indirectly be used to indicate the total scavenging ability of the extract. Majority of the phenolic antioxidants are believed to possess their antioxidant activity by donating a hydrogen atom from its phenol moiety directly or by donating an electron and a proton in a two-step process. However, compounds possessing electron rich aromatic moieties or active methylene groups can act as radical scavengers via adduct formation and/or abstraction of hydrogen atom from the allylic position as is the case with lipids. We have worked extensively and found several phenolic (bakuchiol, dehydrogingerdione, rosmarinic acid, hydroxycinnamic acids etc.) and non-phenolic (folic acid) compounds,which act as efficient *in vitro* antioxidants, following the latter routes. In all the cases, reaction of the ROS with the designated molecules led to the formation of the C-centred radicals. We could show that either the alkyl group or an active methylene group may play a key role in

the antioxidant mechanism if they are present in the side chain of the polyphenols. Using a combination of pulse radiolysis, stable product analysis, biochemical assays and theoretical validation as well as through synthetic congeners, it was possible to rationalize the extent and mechanism of action of herbal antioxidants and their active ingredients.

S052

Oxidative Stress in Cataract and Cardiovascular Disease

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There is strong evidence for the roles of free radicals in a wide variety of diseases and degenerative conditions including pathogenesis of cardiac diseases, cancer and cataract. Oxidative stress may result when the cellular antioxidant defense mechanisms are unable to keep pace with the detoxification of reactive oxygen intermediates. Under physiological conditions, erythrocytes serve the important function of a circulating scavenger of reactive oxygen species (ROS). Erythrocytes exhibit high activities of important antioxidant enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD). glutathione reductase, and catalase. Erythrocyte antioxidant model was therefore chosen to investigate the aspects of cataract and cardiovascular disease. Cataracts develop in diabetics due to accumulation sorbitol within the lens and also with age due to oxidation of lens proteins (crystallins) contributing to senile cataract. The relationship between the presence of cataract and the levels of GSH and G6PD with and without diabetes were investigated. The results revealed that lower levels of G6PD were associated in both diabetic and non-diabetic subjects with cataract. The GSH levels of all subjects with cataract was significantly lower ($P<0.05$) than that of non-cataract subjects.This study was extended to assess the association between the erythrocyte GPX and serum PON-1 concentrations with respect to severity of coronary artery disease (CAD) using vessel, stenosis and extent scores based on coronary angiograms. Results raveled that the PON-1 and GPX activity in patients were significantly (P=000) low compared to control subjects. However, coronary angiography findings revealed that the GPX activity in patients with triple vessel disease was significantly lower (P=0.000) compared to those with the double and single vessel disease. Thus, the erythrocyte GSH and GPX levels appeared to be a more sensitive marker of cataract and PON-1 and GPX to be more sensitive in CAD.

S053

Markers and Mechanisms of Oxidative Stress in Respiratory Diseases

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Chronic respiratory diseases, like asthma and COPD are associated with tissue remodelling in airways and pulmonary blood vessels. Phenotypic alterations in airway smooth muscle cell (ASMC) involving hyperplasia and hypertrophy are the hall marks of airway remodelling. Using immunohistochemical techniques, we demonstrated a number of key markers in the lungs of patients with COPD and compared the expression pattern with non COPD individuals. We found that the expression of several growth factors and cytokines is elevated in patients with COPD and that ASMC contribute significantly in the pathogenesis of disease. To assess the role of ASMC in bronchial angiogenesis and remodelling, we investigated the production of VEGF in ASMC in relation to mediators of asthma, such as, IL-1β, TNF-α, TGF-β, ANG II and ET-1. Time dependent release of VEGF protein in the conditioned medium was observed which in its turn induced proliferation and growth of pulmonary artery endothelial cells. We further investigated the effects of nitric oxide (NO) pathway on Interleukin-1β (IL-1β) induced expression and secretion of VEGF and PlGF from ASMC. Cells were stimulated with IL-1β (5 ng/ml), IL-1β + NO synthase inhibitor, L-NAME, IL-1β + L-arginine for 4 and 24 h. IL-1β induced VEGF mRNA expression was attenuated by L-NAME and augmented by L-arginine that follows by respective protein content in conditioned media at 4 and 24 h, respectively. In another set of experiments, we mimicked the in vitro model of COPD using cyclical strain in ASMC cultured on a collagen coated BioFlex plates. Protein profile using cytokine arrays revealed enhanced stretch induced release of angiogenic molecules; VEGF, Angiogenin, IL-6 and IL-8. VEGF secretion was already higher at 8h compared to controls. Western blot analysis showed robust phosphorylation of ERK1/2 after 15 min and Akt; P-Thr-Akt and P-Ser-Akt after 30 min of cyclical stretch. Respective blockers for Akt, ERK1/2 and Rho pathways revealed significant inhibition of VEGF release only with ERK1/2 inhibitor after 8 h. Taken together, our results suggest that cytokine markers such as, IL-6, IL-8 and VEGF are present in hyper contractile ASMC where nitric oxide pathway may modulate VEGF signalling during airway inflammation and subsequently contributing to bronchial angiogenesis and airway remodelling in patients with asthma and COPD.

S054

Genomic and Proteomic Sciences : Clinical Applications in Infectious Diseases

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Several Scientific contributions made by dedicated researchers led to evolution of genomic era. The term genome was coined by Dr. Tom Roderick a Geneticist in 1986. Confirmation of helical structure of DNA around 1941 by Rosalind Franklin was followed by the publication on the structure of DNA by James D. Watson and Francis Cricks in 1953. Afterwards Frederick Sanger published the amino acid sequence of insulin in 1955. Since then, nucleic acid sequencing became a major target of early molecular biologists. In 1964, Robert W. Holley and his colleagues published the first amino acid sequence ever determined the ribonucleotide sequence of alanine transfer RNA. Extending this work Marshell W. Nirenberg and Philip Leder revealed the triplet nature of the genetic code and they were able to determine the sequences of 54 out of 64 codons in their experiment. In 1972, Walter Fiers team determined the sequence of a gene of bacteriophage MS2 coat protein. Following this, the complete nucleotide sequence of bacteriophage MS2 RNA and a Simian virus 40 was determined in 1976 and 1978. At this time, the DNA sequencing technologies were developed. In addition to his pioneering work on amino acid sequencing Sanger and his colleagues played a key role in the development of DNA sequencing technologies. These technologies enabled the establishment of several genome sequencing projects in 1975, Sanger and Alan Coulson published a sequencing procedure based on DNA polymerase with radio labeled nucleotide that was called plus and minus technology. This technology has lead to Sanger's method which is the basis of DNA sequencing, genome mapping, data storage and bioinformatic analysis most widely used in subsequent years. At the same time, Walter Gilbert and Allan Maxam of Harward University developed the Maxam-Gilbert method, a chemical method of DNA sequencing. The advent of these technologies resulted in rapid intensification in the scope and speed of completion of genome sequencing projects. Complete genome sequencing of a eukaryotic organelle, the human mitochondrion, chloroplast, eukaryotic chromosome, *Sachaaromyces cerevisiae* and *Haemophillus influenzae* were completed. Today complete sequences are available for more than 3,000 viruses and 1500 archaea and 36 eukaryotes of which about half are fungi. Genome sequencing of several important pathogenic microbes were completed and the data is in public domain. The rough draft of human genome was completed in early 2001, completed in 2003, and finished in 2007. Availability of human and microbial genome sequence data has contributed to useful applications of genomics to modern biotechnology, anthropology and social sciences. The genomic technologies ranged from measuring global gene expression to diverse genomic features which include exon level gene expression, DNA binding, single nucleotide polymorphisms and DNA methylation patterns. Micro array technologies and sequencing based technologies are evolved for world-wide applications. The importance of genome is indisputable and paved way to examine the proteome which is essential and responsible for controlling most of the cellular functions. Hence, the study of full array of proteins produced by organisms has become important. In view of this, identification and characterization of proteins became essential. Proteomic technology has been used for better understanding of several infectious diseases such as HIV, AIDS, Tuberculosis, Malaria, Measles, Hepatitis, etc. The benefits so far derived from proteomic and genomic sciences for clinical application can be considered as only the tip of an ice berg. Several break throughs for clinical applications can be anticipated in coming decade.

S055

Proteomics of Genital Mucosal Secretions to Identify the Factors Conferring Resistance to Sexual Transmission of HIV

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Identifying naturally occurring factors, which mediate protection from HIV infection, is critical in the development of effective preventative strategies. Not all individuals sexually exposed to HIV-1 become infected. Evidence from HIV-1 highly exposed sero-negative (ESN) individuals, such as, sex workers and serodiscordant couples, suggests that mucosal factors in the genital tract are playing a role in prevention of transmission. Mucosal factors contain a spectrum of antimicrobials and immune mediators such as Monocyte chemotactic protein-3 (MCP-3), Monokine induced gamma interferon, Semen Derived enhancer of viral infections (SEVI), DMBT1, LL-37, Cathelicidin, Lactoferrin, Lysozyme, Heparin sulfate on the sperm, Secretory leukocyte protease inhibitor (SLPI), α, β- defensins, Mucin, Soluble p-K/Gb histoblood group Antigen, Elafin/Trappin 2, Serpins and Cystatins, that have been differentially expressed in HIV-1 protected individuals.With support from Indian Council of Medical Research and Department of Biotechnology, we have initiated a large scale study to identify the genital mucosal proteome associated with susceptibility/ resistance to heterosexual HIV transmission in a cohort of HIV-1 serodiscordant couples (n=60), established at the Integrated Counselling and Testing Centre (ICTC), Department of Microbiology, KEM Hospital, Mumbai,using iTRAQ proteomics (Multiplexed Isobaric Tagging Technology). The iTRAQ technology uses a chemical tagging reagent which allows multiplexing of two to eight protein samples and facilitates peptide identification and quantitation. The study would identify and validate the human genital mucosal secretome in vaginal fluid,

seminal plasma and urethral discharge of Indian population that contributes to susceptibility/ resistance against the sexual transmission of HIV. Besides, enhancing our understanding of the anti-HIV mechanisms at the mucosal level, these host factors and microflora which can influence HIV transmission could aid in the future development of vaccines, microbicides and therapies.

S056

Proteomic Profiling of *Aspergillus fumigatus* for Understanding the Molecular Mechanisms Involved in Pathogenesis

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Aspergillus fumigatus (*A. fumigatus*) is a saprophytic, ubiquitous and opportunistic fungus, which leads to allergic or invasive aspergillosis in humans depending on the immune status of the host. Proteomic profiling of this medically important fungus may lead to better understanding on the proteins and molecular pathways functional in *A. fumigatus*. We used immunoproteomics approach for identification of novel specific IgE-inducing allergens from culture filtrate of *A. fumigatus* for diagnostic applications in allergic aspergillosis patients. Out of a total of eleven new allergens, three of them viz. a hypothetical protein, extracellular arabinase and chitosanase were major allergens. Proteome profiling of *A. fumigatus* based on two dimensional gel electrophoresis (2-DE) followed by mass spectrometric analysis (MS/MS) led to the identification of 259 unique proteins. We further studied transcriptomic and proteomic profile of *A. fumigatus* on exposure to antifungal agents, amphotericin B and artemisinin, to understand their molecular targets. The expression of genes and proteins belonging to ergosterol biosynthesis pathway and cell wall associated proteins were significantly altered on exposure to amphotericin B, while oxidative phosphorylation pathway was significantly altered on exposure to artemisinin. Some of the regulatory molecules, specific to fungi and belonging to these pathways, are under investigation for their application in drug development for invasive aspergillosis patients.

S057

Survival Mechanisms of Bacterial Pathogens

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Human immune system is capable of fighting most of the pathogens that they encounter throughout their lives. However, several pathogens have the ability to infect the host and establish diseases by modifying the host defence mechanisms, particularly during the initial phases of the infection. Tuberculosis, caused by *Mycobacterium tuberculosis*, is a major global health problem. According to WHO report, tuberculosis takes one life every 22 seconds. Tuberculosis is treated with a combination of four drugs. In the last two decades, multidrug resistant strains of *M. tuberculosis* have emerged. The condition has become graver because no new TB drug has been introduced in the last five decades. *M. tuberculosis* has a myriad of proteins involved in the subversion of our immune system. Similarly, another less known spore forming pathogen is *Bacillus anthracis* which causes anthrax. This pathogen has the ability to subvert the phagocytosis by macrophages which helps in disease establishment. Our efforts are towards understanding the signalling pathways of these pathogens and mechanisms by which they manage to survive in the host.

S058

Proteomics: Tool for the Identification of Novel Drug Targets in Mycobacteria

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Proteomics is a scientific approach used to elucidate the proteome and proteome is the total protein complement of a genome present in cells and/or tissue. Proteomics technologies enable global and unbiased (not restricted to preconceived targets) view of biological systems. With the advent of proteomics science, several biochemical, immunological and molecular mechanisms in human diseases are better understood opening up the scope for development of innovative and specific target based diagnostics. It facilitates the systematic analysis of proteins across any biological system or disease, forwarding new targets and information on mode of action, toxicology and surrogate markers and has been extensively used to tackle a wide variety of medical subjects including biomarker discovery and drug development. The aim of such studies in infectious diseases has been to develop novel vaccines, drugs or diagnostics. *Mycobacterium tuberculosis*, causative agent of

tuberculosis in humans, is the most studied microorganism. Tuberculosis is a serious disease and the TB pandemic has continued despite widespread use of the only available BCG vaccine. Additionally, the increasing incidences of multidrug resistant strains and coinfection with HIV mean that tuberculosis constitutes a growing global threat. Identification and characterization of mycobacterial proteins have been popular research objectives, but comparative proteome profiling of drug susceptible and resistant isolates remain unexplored.Two-dimension gel electrophoresis (2DE) along with mass spectrometry is a powerful tool to study differential protein expression and bioinformatic analysis proves attributive for analysis of diversity of proteins identified by proteome analysis. In-depth study of proteins might give an insight into probable sites of drug action other than established primary sites and hence may help in search of novel chemotherapeutic agents at new sites as inhibitors. These advances are anticipated to result in more meaningful health care to patients in coming years.

S059

Development of Diagnostic test and Vaccine for Tuberculosis: A Viewpoint for the Endemic World

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Of all the global tuberculosis (TB) cases, low and middle income countries account for 95% of TB deaths and one fourth of these cases occur in India annually. The Global Plan of STOP TB for eliminating TB by 2050 primarily depends upon the development of new diagnostics, drugs and vaccines particularly in context of TB endemic countries. In spite of variable efficacy of BCG vaccine particularly in adult TB, it likely will remain a component of childhood vaccination in TB endemic countries because of its beneficial effect in children against miliary TB and TB meningitis. Therefore, a new TB vaccine needs to be proved by sufficient evidence that it is superior to BCG before it could ethically be tested as a substitute of BCG. A prime-boost strategy that includes the benefits of BCG combined with a potent boosting agent could be important in endemic setup. Research being carried out in our laboratory has shown that proteins encoded by genes of "region of difference" (RD) deleted in most or all BCG strains as well as immunodominant epitopes of these RD proteins can be used in combination with BCG in a unique mix and boost strategy to induce protection against experimental model of tuberculosis.In developing countries, diagnosis of TB is primarily based on clinical suspicion, sputum smear examination and /or chest X-ray. Recently, a new automated nucleic acid amplification test Gene-Xpert (GXP) has been endorsed by WHO for TB diagnosis. However, considering the lab infrastructure requirement as well as cost of this assay, it is difficult to implement it as point of care (POC) test in TB endemic settings. Thus, there is need for a robust, rapid and cost effective POC test that can be implemented in microscopy centers as well as in peripheral health care settings. Studies carried out in our laboratory have led to identification of B-cell epitopes of RD proteins that can make the basis for a highly specific rapid POC test for TB diagnosis.Thus, fight against tuberculosis can only be won by developing and implementing the various control strategies particularly vaccines and diagnostics in context of TB endemic countries.

S060

Bioinformatics Approach for Anti-filarial Drug Development

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Human lymphatic filariasis, a parasitic disease affecting mainly developing nations of tropics and subtropics, has imposed profound socioeconomic impediment due to the disability caused.Therefore, WHO has emphasized on validation of novel therapeutic targets considering the gravity of the problem, (under tropical disease research scheme). However, lacunae of knowledge in parasite biology along with difficulty of access to parasitic material from suitable animal model poses formidable challenge. With this perspective, the effective utilization of available genomic and proteomic database in public domain through bioinformatics approach for identification and validation of suitable therapeutic targetswith highly selective criterion specificity might prove to be rewarding. Various schemes for identification of target proteins through wide database search with precise criteria followed by validation with docking of the possible ligands have been deployed for generation of filarial parasite specific myriad databases of potential targets and corresponding virtual ligand database followed by their stepwise logical validation process through cybernetic means, in corroboration with body of evidence generated from wet-lab results. Successful identification and virtual validation of certain unique targets which are essential and non-homologous to human host and also detection of myriad ligand specific targets having growth potential has been accomplished which will be discussed in detail. Also the future direction of mechanistic research will be outlined. Reverse pharmacological approach through bioinformatics not only provide valid therapeutic modality for this parasitic disease but also might develop newer insight into the physiology of parasitic growth process for further in-depth understanding of the disease and host parasite relationship.

S061

Revisiting Clinical Biochemistry in Diabetes and its Complications

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Clinical biochemistry is driven by the discovery of biomarkers, and the availability of appropriate measurement methods. Therefore, its scope constantly changes. Type 2 diabetes is usually diagnosed based on plasma glucose criteria, either the fasting plasma glucose (FPG) or the 2-h plasma glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT). Recently, an International Expert Committee added the A1C (threshold =6.5%) as a third option to diagnose diabetes. The A1C test should be performed using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay. One problem is that HbA1c testing might be biased due to the interference from several hemoglobin variants.Although point-of-care (POC) A1C assays may be NGSP-certified, they should be used with appropriate precautions for screening and diagnosis of diabetes. Recently, the technological advancements also focus on non-invasive point-of-care (POC) devices for population screening and treatment care for diabetes. One of our recent studies used a non-invasive POC medical device that measures the skin collagen fluorescence (as a measure of tissue accumulation of advanced glycation end products, AGEs) and demonstrated its potential in screening for diabetes in the general population. There is also an imperative need and focus on better prediction and diagnosis of gestational diabetes mellitus (GDM) as it caters health care to both the mother and the offspring. Clinically, the first sign of diabetic nephropathy is considered to be microalbuminuria. But we need more sensitive and specific early markers of kidney damage that might help identifying diabetes patients at the highest risk for developing diabetic nephropathy and treating diabetic nephropathy at an earlier stage to prevent the progression to renal failure. Early detection of diabetic neuropathy and disease progression has been limited by a lack of sensitive assessment tools in clinical biochemistry. Early peripheral axonal dysfunction may be detected prior to the development of neuropathy and recent studies imply a role for excitability testing (nerve conduction studies, NCS & total neuropathy score, TNS) in the early diagnosis and testing for the severity of diabetic neuropathy. Studies on different biomarkers of diabetic retinopathy (DR) progression and the identification of different phenotypes of DR with different risks for development of vision-threatening complications also offer new perspectives for understanding DR and for its personalized management. Although at present, it is antivascular endothelial growth factor and antitumor necrosis factor that gain particular significance, we need to look 'beyond VEGF' and several studies are looking at biomarkers of DR and identification of new drug targets to facilitate therapeutic advantage. In recent year, there is also much hype in *'omics'* technologies and *'personalized medicine'* for improving patient care by facilitating interventions tailored to specific subpopulations. New technologies (such as metabolomics, proteomics, genomics, epigenetics) bring a wealth of opportunity to develop new biomarkers.Translating research findings to useful and reliable clinical tests has always been challenging; however, the discovery of ideal biomarkers for diabetes is improving along with the development of biomarker panels and new methodologies. Several studies are under way to develop an integrated biomarker system (IBS) incorporating clinical indicators, metabolites and certain simple 'omics' markers directing towards a powerful tool for biomarker screening for both diabetes and its complications and these will be discussed.

S062

Early Pregnancy Biomarkers for the Prediction of Gestational Diabetes Mellitus

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Diagnosis of Gestational Diabetes Mellitus (GDM) in the very early stage of pregnancy helps to minimize the exposure of the developing fetus to suboptimal conditions and it is invaluable to prevent the maternal and fetal complications of pregnancy. Clinical or biochemical markers for early prediction of GDM are not yet fully established. The development of early biomarkers are specially complicated by the fact that many of the so called GDM cases, in reality, have preexisting diabetes not detected due to lack of organized prepregnancy care. Thus one crucial challenge in early pregnancy is to differentiate between these two groups. This, in turn, requires some evidence on the pathophysiology of diabetes in the specific population. Data from our group suggest that a carefully established cut-off value of simple fasting glucose can be a reasonable tool for this purpose. For prediction of GDM at later stages of pregnancy the roles of many biomarkers like plasma insulin, HOMA%S (as indicator of insulin sensitivity), HOMA%B, sex hormone binding globulin (SHBG), adiponectin, follistatin-like-3 (FSTL-3), platelet count and volume, SNP in L-10 & TNF-α, hsCRP, free beta human chorionic gonadotrophin (fβhCG) and pregnancy associated plasma protein (PAPP-A) have been postulated. We have investigated some of these biomarkers through a series of prospective studies. Various degrees of association of GDM have been found with markers like serum insulin, HOMA%B, HOMA%S, hsCRP (and other inflammatory markers), fβhCG and PAPP-A.On closer analysis it has been found that, so far, PAPP-A is the most reliable early pregnancy biomarker for predicting GDM in our population(with 99% sensitivity, 92% specificity, 93% PPV and 98% NPV). Further studies, however,

are still needed to find biomarker(s) with higher specificity and PPV as well as lower cost.

S063

Global Epidemic of Obesity, Metabolic Syndrome and Diabetes Mellitus

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Aim of this talk is to raise the awareness of the audience of this global epidemic so that timely intervention can prevent its dreaded life endangering complications. Metabolic syndrome, Obesity and Diabetes have almost engulfed the world community in an epidemic fashion! There is a genetic basis but epigenetics play also very crucial role I the expression of this syndrome complex.The understanding and the timely application of laboratory testing in these complex clinical conditions of insulin resistance that leads to life endangering ACS, Vascular strokes and Vascular Claudication remains one of the most difficult problems for clinical laboratory consultants as they are yet to get the laboratory test for the within an hour diagnosis of acute coronary syndrome and or cerebrovascular strokes so that best timely intervention to treat these complex clinical scenario could be done and also there could be many more laboratory tests available to diagnose the development of insulin resistance well in advance so that the occurrence of ACS, Stroke, Peripheral vascular diseases and Chronic Kidney Disease can be prevented. Sedentary life style, induction of stress in life, consumption of alcohol, high glycaemic, fatty and fried food induces Insulin Resistance to set the stage for this global epidemic. This session citing key clinical cases would describe the everyday problems as clinicians in their day to day practice face the rising incidence and prevalence of DM, Obesity and Metabolic Syndrome with the eventual complications and intends to develop a strong awareness to prevent, diagnose and clinically manage of subjects in hospitals and family practices. Genetics and Epigenetics play key roles for the expression of Insulin Resistance syndromes. Appropriate understanding of these syndromes as to their oetiopathogenesis and assimilation of knowledge to develop management strategies including prevention with the timely intervention through the use of appropriate laboratory test repertoire will do the miracle to save the human from suffering from the complications of these syndromes

S064

Differential Functional Deficits in Type 1 Versus Type 2 Diabetes

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Type 1 and type 2 diabetes have different causative mechanisms but the same metabolic outcome, i.e. hyperglycemia. Yet there have been no studies on the differential prevalence of complications in these two types of diabetes. In our experiment, we studied cardiovascular, cognitive and hearing functions in two types of mice - Wild type (WT), Akita and db/db mice (n=6 in each group). For all mice, body weight and blood glucose were measured. To assess cardiovascular functions, blood pressure and left ventricular ejection fraction (LVEF) were measured. Novel object recognition test (NORT) was performed to evaluate cognitive function and auditory brainstem response (ABR) was elicited to assess hearing. Body weight was significantly less in Akita and more in db/db as compared to WT mice. Blood glucose and blood pressure were higher in both types of diabetic mice. Left ventricular dysfunction was noticed in both groups of diabetic mice. NORT calculated discrimination index was decreased in both types of diabetic mice indicating significantly compromised cognitive abilities as compared to WT. ABR threshold was elevated in Akita as well as db/db, but more so in the latter. Blood glucose significantly correlated with blood pressure, LVEF, discrimination index, and ABR threshold, but not with body weight. Hence, we concluded that cardiovascular and cognitive functions were similarly affected in both types of diabetics. On the other hand, ABR threshold was significantly raised in the obese db/db mice, more so than in the Akita, probably due to several concurrent mechanisms affecting the cochlea in db/db mice.

S065

Mass Spectrometric Analysis of Protein Glycation in Relation to Diabetes

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Glycation is a dynamic post translation modification initiated by a non-enzymatic reaction between reducing sugars and amino groups of proteins leading to formation of heterogeneous advanced glycation end products (AGEs). Mass spectrometry provides a powerful tool for characterization of post translational

modifications (PTMs). Various mass spectrometry based approaches such as , label free MS^E, dada dependent acquisition (DDA), high resolution multiple reaction monitoring (HR-MRM) etc, would facilitate PTM characterization. We use some of these approaches for characterization of protein glycation in diabetes. Plasma proteins are the primary target of glycation as they are directly exposed to higher glucose concentrations in plasma. Amongst plasma proteins, albumin is one of the heavily glycated protein and constitutes about 50 % of plasma proteins, and any variation in levels of albumin affects the stoichiometry of plasma protein glycation. Our study has suggested that lower levels of albumin are associated with increased plasma protein glycation and HbA1c, and vice versa. Maintaining higher levels of albumin in diabetes may protect plasma proteins from the adverse effects of glycation in vivo, while lower levels of albumin in diabetes could be risk factor for glycation induced complications. Inhibiting AGE formation has been considered as one of the strategies to prevent glycation induced complications. Further an in vitro MALDI-TOF-MS based insulin glycation assay was developed for screening glycation inhibitors. Using this assay we have discovered strong antiglycation activity for various molecules. The biological effect and the mechanism of action of such inhibitors will be discussed.

S066

Fetuin –A: A Better Marker than IFG for Identification of Pre Diabetes

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Type 2 diabetes mellitus is preceded by a long asymptomatic period designated as pre-diabetes, or insulin resistance. This asymptomatic period if diagnosed at the right time can help the individuals to lead a normal healthy life and can relieve the society as a whole of the burden of treatment of diabetes mellitus. Keeping in view the magnitude of incidence of pre-diabetes, the present study was planned to study fetuin- A, and find its correlation if any with various biochemical investigations used as markers of pre- Diabetes or IR. A total of 742 young individuals were recruited for the study. These individuals were offsprings of diabetics and were siblings amongst themselves belonging to age group of 18-35 years. Various biochemical investigations such as fasting plasma glucose, Glycosylated Hb, S. insulin, C-peptide and fetuin-A, apart from anthropometric measurements were carried out. The results of the present study depicted that with advancing age the percentage prevalence of diabetes increased both in males and females.Hyperglycemia and Hyperinsulinemia with corresponding increase in the levels of C-peptide clearly demarcated the individuals with insulin resistance. Serum Fetuin-A concentrations were 330.82± 15.4, 654.5±16.3 and 1046.7±17.34 μg/ml in normal, IFG and diabetic individuals respectively. Regression analysis showed Fetuin A concentration was positively associated with fasting plasma glucose, Insulin, C-peptide, and IR and was negatively correlated with beta cell function. Variations in fetuin-A levels were independent of age. A positive correlation of Fetuin-A with the various biochemical investigations suggests that fetuin-A can be used as a tool for detecting pre diabetes and also the susceptibility of an individual towards insulin resistance. Fetuin –A being a stable component can be a better parameter for identification of pre diabetes.

S067

Molecular and Functional Basis of Cystic Fibrosis: Diagnostic and Therapeutic Implications

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Cystic Fibrosis an autosomal recessive disorder is usually considered as rare disease for Indian population hence much less is known about this disease in context with Indian sub-continent. Recently we have established a spectrum of mutations in CFTR gene from classical Cystic Fibrosis as well as from infertile male patients with CAVD in Indian populations. Among them S549N, L69H in classical CF and infertile CAVD males and G126S, F87I, S118P, H139Q, F157C, F494L, E543A, Y852F, D1270E only in CAVD males were among the rare missense mutations. In this study we have attempted to conduct in vitro gene expression analysis to establish genotype and phenotype correlation and to characterize these four rare missense mutations according to the mechanism that disrupt CFTR protein function. All eleven mutations from Indian population were characterized by expressing pEGFP-CFTR constructs in BHK-21 cells using 3 step technique viz; CFTR cellular localization was determined by confocal microscopy, whereas Western blot analysis and automated iodide efflux assays was used to determine CFTR maturation processes and its chloride channel activity respectively. In Western blot analysis only immature core glycosylated CFTR 'B'-band is obtained for L69H mutation similar to F508del mutation whereas in the case of other mutants both 'B' and 'C' bands were found, indicating L69H mutation impair CFTR maturation process, the finding was again confirmed by confocal imaging. Iodide Efflux assay revealed significant decrease in channel activity for L69H and S549N mutants CFTR expressing cells in comparison to WT, although this decrease in channel activity was rescued when cells were incubated at 27°C. When the effect of CFTR correctors was checked on different mutants, it was found that VX-809 significantly ameliorate the defect caused by L69H mutation. Mutations G126S, F87I, S118P, H139Q, F157C, F494L, E543A, Y852F, D1270E have no impact on CFTR maturation and function. Thus we can conclude that L69H mutation is a class II CF causing mutation causing impaired maturation leading to protein degradation and Cl^- ions

impermeability as observed in F508del mutation. This defect is rescued by the corrector VX 809. Whereas S549N mutation can be categorized into a class II/III mutation causing impaired maturation and reduced channel activity.

S068

Role of Pharmacogenetics in Clinical Practice

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There is considerable inter-individual variation in response to drug therapies. Some patients may not respond while others may develop toxicities for similar drug dosage. Part of such disparities has been attributed to genetic variations in key genes involved in drug metabolism, transport and drug targets. Over the last few years, we are trying to dissect out genetic factors in number of diseases such as epilepsy, anti-coagulant therapy in thromboembolic conditions, and chemo-radio-therapies for breast and gallbladder cancer (GBC). Multiple drug resistance in epilepsy is a common problem and almost one third of epilepsy patients remain non-responsive to antiepileptic drug (AED) therapy. Such inter individual variations in drug response are believed to result from genetic variations in candidate genes belonging to multiple pathways. Overall, our results demonstrated significant involvement of CYP2C9, SCN1A, SCN2A genetic variants in the modulation of epilepsy pharmacotherapy and differential role of different subunits of GABA(A) receptor subtypes in epilepsy susceptibility and pharmacotherapy.Coumarinic oral-anticoagulants (COAs) are commonly used for treatment of thromboembolic events. Dose requirements for oral anticoagulants are influenced by polymorphisms in VKORC1, CYP4F2, GGCX, CYP2C9 and APOE genes. However, the final drug dosage in an individual is determined by complex sets of genetic and environmental factors. Several dosing algorithms which combine clinical and genetic parameters to predict therapeutic COA doses have also been developed which are population-specific. We have developed a North-India specific algorithm for the pharmacogenetic prediction of drug-dosage in oral anticoagulant therapies.Various combinations of chemotherapeutic drugs form an integral part of the systemic treatment of breast cancer patients. However, there is a large heterogeneity in the response to, and toxicity of, chemotherapeutic agents in breast cancer patients. Therefore, we studied the correlation of several polymorphisms in Phase 0, I, II and III metabolism pathway with drug treatment outcomes. We found ABCB1 1236C>T polymorphism to be statistically significant with response to NACT and grade 2-4 anemia. We also found significant association of *1/*3 of CYP2C9*3 with grade 2-4 leucopenia (P=0.040) and CT genotype of NQO1 polymorphism with dose delay/ reduction (P=0.021). Gemcitabine based chemotherapy is the main treatment of patients with GBC. Till date, there is no study on gallbladder cancer chemotherapeutic treatment outcomes. Therefore, we carried out a pilot studyfor the SNPs based on genes (CDA, dCK, RRM1, DCTD, hCNT2, hCNT3, hENT1) involved in Gemcitabine metabolic pathway with response to treatment, chemo-toxicity and overall survival. We also evaluated our prognosis data with microRNA polymorphisms and found that variant alleles of miR-27a and miR-181a were associated with poor therapeutic response in GBC. In addition to these examples, there is large number of other drugs whose safety and efficacy is significantly influenced by genetic profile of host. As newer pharmacogenetics/pharmacogenomics information becomes available, its association with the safe and effective use of drugs is being incorporated in the routine practice. However, there are still in-numerable challenges which need to be addressed before translation of genetic information to product labeling and clinical practice.

S069

Practical Guidelines for Setting up Diagnostic Facility for Inborn Errors of Metabolism

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Inborn errors of metabolism, IEM (also known as inborn metabolic disorders, IMD) arise due to defect in single enzyme in metabolic pathways. IEM are individually rare, but collectively they form an important group of disorders. It is therefore important that laboratory facilities should be available for diagnosing IEM. A basic screening laboratory can have simple urine screening tests. Amino acids in urine can be screened by ninhydrin test followed by other color reactions and thin layer chromatography. Sugars can also be detected by simple tests. The service of the clinical biochemistry is very essential for a basic screening laboratory. Investigations like blood glucose, ammonia, anion gap and lactate give very important clues for diagnosing IEM. A referral laboratory should have advanced facilities like high performance liquid chromatography (HPLC), gas chromatography/mass spectrometry (GC/MS) and tandem mass spectrometry. HPLC is very useful for detecting aminoacidurias and organic acidurias, and can also be used for acyl carnitine profiling for detecting fatty acid oxidation disorders. GC/MS is however more useful for detecting organic acids and acyl carnitine profiling. Tandem mass spectrometry is used in Western countries for newborn screening, however in India very few laboratories can afford the cost involved. However, it is very useful for screening high risk children. It can detect more than 50 disorders from a single heel prick sample. Sophisticated instrumentation is not everything; biochemical tests are also important. Disorders like galactosemia, phenylketonuria, congenital adrenal hyperplasia and congenital hypothyroidism are detected by enzyme based biochemical tests even in advanced laboratories.

S070

Newborn Screening in India: Early Intervention and Management of Inborn Errors of Metabolism

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Inborn errors of metabolism comprise a group of disorders which if not treated on time may lead to death of the neonate. Detection of metabolic disorders in asymptomatic phase is only possible through the blood spot test popularly known as newborn screening. Newborn screening (NBS) programs are running successfully in the developed countries and have saved million of newborns. In India 5-15% of sick newborns have metabolic disorders, but due to lack of awareness and infrastructure burden of metabolic ill newborns is increasing day by day. In India newborn screening programs are still confined to few states and government funded projects. In Chandigarh at Government Medical College and hospital newborn screening program was started in 2007 as a component of "prevention of disability act''. After reviewing the literature three disorders were selected in the NBS panel namely Congenital Hypothyroidism, Congenital Adrenal Hyperplasia and G6PD deficiency. Sample for testing are collected through heel prick on a special graded filter paper. Till date we have screened around 28,000 newborns, and by using various strategies we have now have developed a model to extend this facility to other hospitals of UT and to implement this program as a model for India.

S071

Distribution Pattern of Ghrelin Gene Polymorphism

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Ghrelin is an orexigenic hormone and may influence the development of obesity through its role in control of energy balance. In the present study, it was decided to study the single nucleotide polymorphism (SNP), rs35683 (G62T) in ghrelin gene in obese and normal individuals.Objective of the study wasto study a SNP in the intron region of ghrelin gene in normal and obese individuals. The study population of age group (18 to 40 years) were divided into two groups based on BMI (18.5 – 22.9 kg/m^2) as Group I, n=196 and BMI = 25 kg/m^2 as Group II n= 180. The study population included normal healthy individuals without any known or reported health problems. The tagging SNP (rs35683,G62T) in ghrelin gene was selected using genotyping data available on Gujarathi Indians in Houston. The DNA was isolated from EDTA blood by proteinase K method. The DNA samples were analyzed for SNP by TaqManSNP methodology. The genotype distribution of the polymorphism G62T in the intron region of ghrelin gene was tested for Hardy-Weinberg equilibrium and the data was analysed by Chi- square test.The SNP in obese and normal were found to be in Hardy -Weinberg equilibrium. Chi-square analysis showed a significant difference between the three different genotypes in Group I and Group II.The SNP (rs35683,G62T) is likely to have some influence on obesity in this study population.

S072

Neonatal Screening for Congenital Hypothyroidism: A 9 Year Experience at a Tertiary Care Centre

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Congenital hypothyroidism (CH) is insufficient thyroid hormone production in newborn infants and is one of the most common preventable causes of mental retardation. It is due to defect in thyroid gland development (dysgenesis) or a disorder of thyroid hormone biosynthesis (dyshormonogenesis). Identify infants with congenital hypothyroidism with the goal of reducing the effect of the condition on the child through earlier treatment. A drop of blood specimen was taken from the heel after the first 48-72 hours of life in S & S 903 filter paper. TSH was analysed using Bio-Rad screening kit in the laboratory (2005-2010). From January 2011 to September 2013 we used rapid quantitative immunoassay from veda lab, France in the neonatal ward and from October 2013 we use PerkinElmer Time-resolved fluoroimmunoassay. All the positive cases were reconfirmed by 3rd generation TSH kit as well as FT4 in chemiluminesence assay. Since the introduction of routine screening for congenital hypothyroidism we have screened so far 22,039 newborns over the past 9 years, 14 had CH (incidence 1:1574). In view of the high incidence, apparently asymptomatic nature, propensity to cause neurodevelopmental delay, early detection and treatment of CH would be the most cost effective method to confront this problem. However, newborn thyroid screening is not yet universal in our countries. It is high time to start routine neonatal screening for CH to tackle this preventable cause of mental retardation and to estimate the true extent of the inborn errors of metabolism.

P073

Quality Management:Integration of Error Disclosure in Health care and Laboratory Medicine

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In any health care process, adverse events resulting from errors are inevitable. The issue of medical error has received substantial attention in recent years. 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. Disclosure of an adverse event is an important element in managing the consequences of a medical error. 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, Canada, New Zealand and United States of America). In Australia, disclosure policy integrates the disclosure process with risk management analysis towards investigating the critical events. 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 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. We believe the top priority of healthcare providers should be focused on correcting flaws in the medical system and the subsequent protection of patients' health. Despite the obstacles, physicians should seek to disclose medical errors to patients and their families on both ethical and pragmatic grounds. Effective communication between health care providers, patients and their families throughout the disclosure process is integral in sustaining and developing the physician patient relationship. A uniform policy centered on addressing errors in a non-punitive manner and respecting the patient's right to an honest disclosure should be a standard of care.

S074

Parallel Processing of Internal Quality Control Samples to Monitor Inter-Instrument Comparability

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With increasing work-loads of clinical biochemistry laboratories, it has become inevitable to have more than one analytical equipment with overlapping test menus. Samples are routinely processed simultaneously on two instruments in order to facilitate rapid reporting of results. In such a situation it is imperative to establish comparability of results between them. This study sought to use results of assayed quality controls to monitor inter-instrument comparability. The study was carried out on two automated clinical chemistry analyzers Cobas Integra 400 plus based upon liquid reagents (Roche) and Vitros 250 which makes use of dry slide technology (Ortho Clinical Diagnostics). Two levels of Bio-Rad assayed controls were run simultaneously, daily for a period of 90 days for routine parameters. The inter-instrument bias, calculated as a percentage was found to be within acceptable limits for the above parameters. The Levey Jennings plot had values distributed within the 2SD limits for both instruments indicating that precision was good for both the instruments. Any deviations could be detected prior to processing of patients samples in order to enable timely institution of corrective actions actions.Parallel running of assayed controls is an ideal method that could be used to verify inter-instrument comparability. It demonstrates on-going acceptability of results generated, which in turn captures significant changes in the results of patients being monitored for their therapeutic response. Vitros was chosen as the second instrument as it has the advantage of not requiring water, the quality of which is difficult to maintain and monitor.

S075

Quality Assurance in the Molecular Diagnostics: Practical Approaches for Addressing Day-to-day Challenges in a Clinical Laboratory

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S076

Integrating while Specialising the Laboratory: Expanding to Occupational Health

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Integration of medical laboratories has been trigerred by patient safety and improved general care issues, customers' needs for one point service, technology developments, but mostly by efficiency of resources issues. Laboratories have widened their service comprising non-laboratory operations, and implemented informatics and automations from the very simple to the mostly automated robotic systems. While integration has become indispensable for better patient care and economic survival, medical science has developed discoveries in its diverse disciplines: occupational health, women's health, children's health, geriatrics, to mention some, which challenges the medical laboratory to synergise for the benefit of the patient and community. One of the new specialization which apparently needs integration of operations from the medical laboratory side is occupational health, which focuses on the wellness of workers particularly wellness as required to fit to specific occupation. The issues in occupational wellness and freedom from occupational hazards is to be 'fit for work', not just normal in the general understanding of reference values. The medical laboratory which provides services for occupational health needs to have not only general medical laboratory testing services, but also specific occupational, environmental testings, as well as occupation-specific testings which are of non-laboratory nature. The functioning of an optimal occupational medical service provides assurance for better workforce productivity.

S077

Emergence of Laboratory Medicine Old Wine Enriched In a New bottle!

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Laboratory Medicine as a discipline has grown from the 'side lab' concept to 'Point of Care', and in between a large central hospital laboratory (originally clinical pathology laboratory), which delivers report with Turn Around Time (TAT) of 2-8 hours. Laboratory Medicine in its present phase includes investigations from the recognized discipline of Clinical Pathology, Clinical Microbiology, Clinical Hematology, Clinical Biochemistry, and Clinical Immunology and Molecular diagnostics in the central laboratory of a tertiary care hospital or a multidisciplinary diagnostic laboratory. Because of enormity of investigations which have been recently shifted from research premise to a clinical diagnostic laboratory, because of growth of automation starting from sample collection to laboratory analysis and laboratory information system with elimination of common human errors and development of defined methods of quality assurance on accuracy and precision of the investigation result, the old wine of all–inclusive Clinical Pathology which did establish the initial foundation for evidence-based medical practice has come up enriched in a new package, in a new bottle with a charming label known as the Discipline of Laboratory Medicine. As a consequence, there is a felt need of reorganization of medical laboratories and production of all-rounder post-graduate specialists who can take care of all of the above investigations with the basic undergraduate knowledge of Medicine. This warrants a total recasting of the diagnostic disciplines like Clinical Biochemistry, Hematology, Histopathology and Microbiology as super specialty DM course following completion of the basic post-graduation residency in Laboratory Medicine. When research and publications are part and parcel of any diagnostic laboratory offering doctorate degree, scientists with experience of having a Ph.D. degree in the related disciplines should also be accommodated as Faculty in specific and selected areas where research and publication have priority over immediate patient care.Emergence of Laboratory Medicine as a discipline of its own concurs with Three Tier Concept of a Medical Institute's Laboratory; the Tier I constitutes the discipline of Laboratory Medicine, Tier II constitutes the super specialty laboratory of Histopathology, Microbiology, Hematology, Biochemistry, Molecular Biology and Immunology and Tier III remains the high-end common research facility laboratory, the pride laboratory of the Institute/Medical Centre.

S078

Validation of Sample Preservation Policy of Routine Clinical Chemistry Parameters

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The recommendation for preservation of primary samples for Clinical Chemistry as per ISO 15189:2007 & 2012 is 24 hrs & and to be preserved in aliquot at 2°-8°C immediately after completion of analysis for additional/repeat testing. But from sample collection to processing, there is an on the table time lag and preservation immediately after completion of test is by all practical possibility a difficult task. Hence, it was decided to observe the deviation of routine clinical chemistry examination results with time when preserved in primary sample container. Primary samples are collected in evacuated container without gel separator. 17 Routine parameters were retested at a time lag of 4hrs at room temperature (22°C-25°C). On 2nd phase, the time lag has been increased to 6-7hrs keeping temperature factor constant. On 3rd phase, the samples were kept at room temperature for 6-7hrs, and then preserved at 2°-8°c in primary sample container overnight and retested. The

deviation between two results and regression has been calculated. Upto 5hrs all parameters shown good regression factor. After 24 hrs only T.Protein, T.Calcium,Inorganic Phosphorus and Albumin failed regression criteria. Except above mentioned four parameters all other routine parameters may be preserved in primary sample container overnight for retesting/additional testing provided room temperature is ambient and primary container is of good quality. The preservation policy is validated subject to maintenance of present system. Any change would require revalidation.

S079

Applications of Stem Cells in Treatment of Malignancies

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Stem cells are used in malignancies for treatment mostly in relapse and refractory malignancies. Allogenic transplantation is used wherein primarily the malignancy involves the stem cells. Autologous transplantation on the other hand is utilized as a rescue wherein high doses of chemotherapy are administered to patients in a relapse setting. Notably only 30% of patients who require an allogenic transplantation have a matched sibling donor. Thus in these situations, the alternatives include matched unrelated donor and/or cord blood stem cell transplantation. Presently, there is also emerging data on the use of haplo-identical transplantation, although the risk of graft versus host disease and added immune suppression resulting in viral reactivation particularly CMV infection is still a major issue. The pros and cons of all these modalities as well as the experience from AIIMS will be discussed.

S080

Cervical Cancer and HPV Infection: Targeting Cancer Stem Cells by Herbal Anti-cancer Derivatives

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Persistent infection of high risk Human Papillomavirus (HPV) is causally linked to the development of cervical cancer. Expression of HPV viral proteins E6 and E7 that are transcriptionally regulated by a set of host transcription factors such as AP-1, NF-κB and STAT3. These transcription factors are key oncogenic mediators and serve as therapeutic targets. Over last decade, our laboratory has discovered potential anti-HPV, anti-cancer agents like curcumin and berberine that mediate their activities through inhibition of these transcription factors with variable efficacies. The heterogeneous nature of tumor and presence of cancer stem cells are considered as main obstacle that reduce the effectiveness of these and other anti-cancer therapeutics. However, CSCs are poorly characterized in cervical cancer and role of viral oncoprotein in the maintenance of the CSC phenotype yet an unexplored area. We have identified and characterized CSCs from HPV positive cervical cancer cells on the basis of functional and stem cell markers. Cervical CSCs were able to maintain their proportion in the presence of standard chemotherapeutic drugs such as 5-FU and resulted in upregulation of stemness related transcription factor such as GLI which functionally contributed to the observed chemoresistance. These CSCs expressed differentially activated stem cell signalling pathways such as Notch and Hedgehog and stemness-related transcription factors such as Oct 4, Nanog and Sox2 and demonstrated a specific HPV oncogene expression profile. Furthur, studies are needed to develop these transcription factors and CSC signaling mediators as therapeutic targets for better clinical outcome against HPV infection and cervical cancer.

S081

Sensitization of CML Blasts Cells to Imatinib Mesylate Therapy by Cathepsin L Over Expression

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Cathepsin L (CTSL), a lysosomal cysteine protease secreted by malignant cells plays an important role in tumor invasion and metastasis. The expression and significance of cathepsin L has been extensively studied in solid tumors. However no such information in chronic myeloid leukemia (CML) was available. We investigated the activity and expression of this protease in peripheral blood mononuclear cells (PBMCs) of 47 adult CML patients. 30 adults suffering from systemic diseases and 50 healthy volunteers served as controls. The mRNA levels of CTSL, its specific endogenous inhibitor cystatin c and transcriptional up-regulator VEGF were quantitated by Real-Time qPCR. CTSL protease activity and its mRNA expression were significantly high in CML chronic phase (CP) patients compared to CML accelerated phase/blast crisis (AP/BC) and controls (P=0.001). VEGF whose expression was most pronounced in CP and declined (P=0.001) in the advanced phases of the malignancy exhibited a strong positive correlation with CTSL

(r=0.97; P=0.001). Cystatin C expression was significantly lower (P=0.001) in CML and displayed inverse correlation with CTSL (r= -0.713; P=0.001) activity. CTSL promoter was significantly hypomethylated in CML CP compared to CML AP/BC patients as well as controls. K562, a blast crisis CML cell line displayed CTSL activity, expression and methylation status of CTSL promoter that was comparable to CML AP/BC patients. Treatment of these cells with 5'-aza-cytidine resulted in a dramatic increase in CSTL activity and expression thereby demonstrating the role of promoter methylation in the stage specific expression of CTSL in CML. Stable transfection of K562 cells with CTSL expression vector resulting in the over expression this protease significantly increased their sensitivity to imatinib mesylate thereby suggesting the role of CTSL in chemo-sensitization of AP/BC leukemia cells which are otherwise resistant to chemotherapy.

S082

Hypoxia on Notch Signalling, Epithelial-mesenchymal Transition (EMT) and Stemness in Glioblastoma

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Hypoxia is a critical feature of all solid tumors including glioblastoma. It promotes tumor progression, invasiveness and stemness. We quantified hypoxia/Notch/EMT/stemness genes in 35 primary glioblastoma tissues by q-PCR and immunohistochemistry. Statistical analyses were employed to identify hypoxia markers associated with upregulated Notch pathway/EMT/stemness genes. Hypoxia-mediated response of Notch pathway/EMT/stemness was also analyzed in U87MG gliomasphere under low oxygen concentration (2%/ and 0.2% O_2). We identified a hypoxia-Notch axis in glioblastoma (HIF-1α/PGK1/VEGF/CA9/OPN-Notch1/Dll1/Hes1/Hes6/Hey1/Hey2). Similar response was also identified in hypoxia-exposed U87MG gliomaspheres, supporting them as an *in-vitro* model for elucidating hypoxia-Notch association. Glioblastoma aggressiveness under hypoxia was also found to be contributed by Snail which correlates with upregulated stemness markers. We found HIF1α, OPN and Hes1 to be prognostically relevant in GBM patients.

S083

Pharmacogenomics: An Indian Perspective

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It is well known that the inter-individual genetic variations that lead to many phenotypic variations play an important role in altering the drug response. The field of pharmacogenomics which offers personalized medical healthcare makes use of various genetic screening tools to identify these genetic variants amongst individuals in order to improve drug safety and optimize its efficacy. There have been many pharmacogenetic studies in India that have attempted to determine the frequency of variant allele of drug metabolizing genes (Cytochrome P450s superfamily) which has observed to be quite different from the other populations. In India, the knowledge of pharmacogenomics has been applied in various clinical situations such as chemotherapeutic drugs administration for cancer patients, use of warfarin for treatment of thromboembolic diseases, use of immunosuppressive drugs to prevent drug induced nephrotoxicity and improve graft acceptance rate etc. 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 would be the variant allele frequency. 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 increasing understanding due to high throughput technologies of the genetic susceptibility along with the greater interest / support from the government agencies like DBT, DST and ICMR together shows a lot of promise in the future in favour of personalized healthcare in India.

S084

Monitoring of Immunosupressive Drugs in Renal And Liver Transplant Patients

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Immunosuppressive drugs (ISDs) form the mainstay of treatment following organ transplantation, ensuring long-term graft survival and improving the overall success rate of transplantation. Rejection may occur at any time post transplantation, and hence the lifelong administration of ISDs is usually required. The treatment regimen necessitates the regular monitoring and regulation of ISD doses in order to prevent probable rejection events and major adverse effects. The most commonly used ISDs are cyclosporine, tacrolimus, sirolimus, mycophenolic acid, azathioprine, metlylprednisolone etc.

The monitoring of cyclosporine or tacrolimus is always recommended due to their narrow therapeutic index. In present study we have followed 5 patients for 90 days and measured cyclosporine, C_O (trough levels) C_2 (2 hours after dose) levels by using Emit Syva kit (Spectrophotometry). The levels were measured on 4th, 7th, 15th, 30th, 60th, and 90th day of renal transplant (RTx). The C_2 level remained in the range of 1.5-1.7 micrograms/ml for the first month, in second month it was 1.39 and third month it reached to 1.17 when dose decreased from 250 mg BD to 175 mg BD, the mean creatinine remained at 1.5 mg/dl. Whereas C_O level remained unchanged for 3 months with mean of 0.35 μg/ml. This study shows Cyclosporin C_2 level is better than C_O level for monitoring drug dose in renal transplant. Achieving target of 1.75 μg /ml within 7 days of transplant of C_2 limits the occurrence of acute rejection. For the tacrolimus study involved retrospective analysis of 15 RTx patients who were on triple immuno suppressive therapy, methyl prednisolone, mycophenolate mofetil and Tacrolimus. Blood samples were collected before administering Tacrolimus (0 h) to determine trough concentration and at fixed time points of 2h, 4h and 6h after administration of oral Tacrolimus and analysed induplicate by microparticle enzyme immunoassay. AUC_{0-6} was determined using the linear trapezoidal rule. The trough levels were fairly consistent at 7.9-18 ng.h/mL in all the patients included in this study and this did not show variation with age or sex. The AUC0-6 was higher (202-290 ng/mL at 3-8 mg BD dosage) in patients who received kidney from cadaver compared to recipients from live donors (60.5-171 ng/mL at 3-8 mg BD dosage) but whether the clinical significance of this is not known. Highest AUC0-6 was 246 ng/mL observed at 4.5 mg BD dosage. Dosages higher than 2 mg BD did not result in noticeable increase in AUC_{0-6}. Peak blood levels of tacrolimus were obtained 4 h after administration to conclude, trough level determination and C2, C4 two-point limited sampling strategy may be useful to plan the dosing strategy and estimate exposure of RTx patients to tacrolimus.We followed 4 patients for 94 days and measured trough levels of tacrolimus on 4th, 10th, 17th, 21 st, 25 th, 31 st, 34 th, 52nd, 64th and 94th day of post liver transplant (LTx). Urea and Creatinine were also estimated. These patients received 0.5 mg BD prograf as starting dose on 2 nd day of LTx. Due to cover of IV methylprednisolone, (1 gm I.V. started at anhepatic phase and 20 mg/day for 4 days and will be reduced to 5 mg/day by the end of one month) chance of ACR is little. Flexible dosing in the early period, post LTx so that by day 5 (when metlylprednisolone is withdrawn) levels of 10-12 ng/ml is aimed. Mycophenolic acid 500 mg BD will be given till renal parameters becomes normal. At the time of discharge from index hospitalization, aim of tacrolimus levels close of 7-9 ng/ml with minimized doses prograf will be maintained to prevent acute cellular rejection.

S085

Drug of Abuse and Toxins Testing

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The wide spread illicit use of several drugs and substances has made testing for toxins a challenge. Added to this list is the continuous polluting with environmental toxins: mutagens/ carcinogens and heavy metals which accumulate in the body with deleterious consequences. Clinical Toxicology is the measurement and interpretation of concentrations of drugs and other toxic substance in human biological fluids for the purpose of patient care. Toxicology screening helps the clinician to narrow down the diagnosis and provide quick, effective therapy. Qualitative analysis by screening the urine is still the order of the day and is used in emergency situations to detect intentional or accidental overdose of prescribed, non-prescribed or illicit drugs. Screening procedures involve immunoassay and chromatography techniques and can detect the drug or metabolic derivatives and are reported based on established cut-off values. Also it is slow cost, easily accessible and very convenient for rapid screening. Other screening techniques include spot urine test and spectrochemical test. Rapid homogenous Immunoassay techniques include: Enzyme mediated immunologic technique (EMIT), Flourescence Polarization Immunoassay and Radioimmunoassay. But they have to be confirmed by sensitive and refined techniques such as Gas chromatography-Mass spectrometry (GC-MS), High Performance Liquid chromatography (LC-MS). Source of the biologic specimen affects the characteristics of the testing. False positive and false negative reports need careful scrutiny of drug history for interpretation. Pharmacokinetics and individual variations in handling substances play a great role in their detection. Hence blood specimens are not routinely used for quantitation. Saliva, sweat and hair are the other specimens for use in toxin testing which differ in their characteristics and periods of detection. Sweat and saliva have shorter detection times and can detect even trace amounts using electronic immunoassay technique but methods of collection and transport has made them less feasible. The biochip array technology using competitive assays for multiple drugs is the latest in rapid diagnosis. Ethanol, Methanol, organo-phosphorus poisonings, rat poison and hair dye ingestion is the commonly encountered toxins in the Indian scenario. Carbon monoxide and lead poisoning are among the other causes of toxicity. Enzymatic, Spectrophotometric, electrochemical oxidation, estimation of metabolites are the methods to assess toxicity.

S086

Therapeutic Drug Monitoring and Pharmacogenomics

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Therapeutic Drug Monitoring (TDM) is a branch of clinical chemistry that specializes in the measurement of drug levels in serum. TDM requires that the laboratory make quantitative measurements of drugs and/or their metabolites. Providing accurate concentrations for TDM allow clinicians to adjust drug dosages as well as assess a patient's drug metabolism response and their compliance or dosing regimen. TDM is the clinical assessment of a drug's pharmacokinetic properties. Physicians often need to establish that a drug is present at a clinically-effective concentration yet does not reach a toxic concentration. Some drugs have a very narrow therapeutic window and need closer monitoring. Drugs with narrow therapeutic windows should be closely monitored since elevated doses can cause serious conditions such as agranulocytosis. Four major classes of drugs are frequently monitored by TDM are Antibiotics, Anticonvulsants, Immunosuppressants, and Cardiac drugs. TDM helps to ensure that a dosing regimen is appropriate for a given patient. Immunoassays are the most common technique used by clinical laboratories for therapeutic drug monitoring. Some of these methods are Particle-enhanced turbidimetric inhibition immunoassay (PETINIA), Cloned enzyme donor immunoassay (CEDIA), Fluorescence polarization immunoassay (FPIA), and Chemiluminescence immunoassay (CLIA). Pharmacogenomics (PGx) is the study of how individual variations in the human genome affect responses to drugs. It refers to how administered drugs will be handled by a specific person given specific genetic mutations and polymorphisms they may have. The primary reason that individuals metabolize and respond to drugs differently is the inter-individual differences in receptor proteins and enzymes that metabolize the drugs. Mutations in these receptor proteins and enzymes can give rise to very different responses to drugs. In PGx, these mutations are referred to as variants. A polymorphism is a variation in a specific gene (allele) that affects at least 1% of the population. It is essentially a mutation that occurs relatively frequently in the population. CYP450 refers to a family of enzymes found predominantly in the liver. CYP450 enzymes work on a variety of drugs, altering their chemical structures to facilitate excretion in the urine and feces. The major subfamilies of CYP450 enzymes that have to-date, been associated with significant polymorphisms that affect drug disposition are CYP1A2, CYP2C9, CYP2C19, and CYP2D6. Recent estimates show that 6-10% of the general population have a complete deficiency of CYP2D6, with the prevalence of mutations varying from <1% to as much as 21% within a given population. The ultimate goal in measuring CYP450 function or identifying polymorphisms is to predict effective therapeutic doses and responses in patients. Phenotyping involves measuring the metabolism of a probe drug, referred as 'probe drug testing'. Unlike genotyping and probe-drug testing, therapeutic drug monitoring must be performed during therapy, not before. So, in fact, TDM is not really used to predict therapy in PGx but serves as a confirmation of PGx findings. TDM and genotyping should be considered complementary and can be used in tandem to, first, predict and then verify appropriate serum drug levels. Polymorphisms are identified using molecular techniques like allele-specific PCR, restriction digests, sequencing, hybridization assays, bead-based systems, microarrays, and pyrosequencing.

S087

The Uncertainty of the eGFR Equations

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Assessing renal function cannot be performed without laboratory testing. Traditionally the gold standard of renal function was to measure glomerular filtration rate (GFR) using a clearance test but the only practical analyte available was creatinine and the creatinine clearance has been used to measure GFR. However there are many practical issues with the performance of this test and it is rarely used. By default serum creatinine and urea have been the mainstays of renal function testing but they have significant limitations because of poor sensitivity and specificity for renal failure. To overcome the problems with serum creatinine which relate to variation in the production of creatinine because of muscle mass differences in males and females as they age the estimated GFR or eGFR has been introduced. This is a function which produces a GFR based on the serum creatinine, age, sex and ethnicity. There have been a number of different eGFR formulae produced MDRD and CKD-EPI and each has its limitations. These are because of the way the model is 'fitted' to the data and the coefficients for ethnicity used. It is now becoming apparent just how significant these differences are in terms of the calculated eGFR. With any of these though there is an associated measurement uncertainty which becomes important around critical decision points which are related to the definition of chronic kidney disease. Perhaps the greatest uncertainty is when the eGFR is close to 60 mls/min/surface area which can be when the creatinine is within the reference interval. These are the creatinine levels which are measured with greatest uncertainty by laboratories. The value of an eGFR in ascribing risk in chronic kidney disease is now also being questioned and it is suggested that urinary albumin should also be used to assess risk. Thus the uncertainties of the eGFR are many and include the error of the creatinine, the appropriateness of the equation, are the ethnicity coefficients in the equation the correct ones and how well does the eGFR identify at risk patients.

S088

Analyses of Hemoglobin Variation Among Acute Care Patients Through Data Mining: Walking the Path to Integration of Laboratory Medicine

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Anemia or low hemoglobin (Hb) level is predictor of morbidity and mortality among all acute care patients. Unexpected variation of Hb levels in hemodialysis patients resulted in unnecessary RBC transfusions and triggered a laboratory practice audit. Objective was to evaluate the scope of Hb variation and elucidate root causes of Hb variation among hemodialysis patients. Records provided weekly pre-dialysis Hb levels for patients for one year, (n= 300 patients) with patient identifiers and dates. Quality control data was extracted. Hb was determined by Beckman Coulter LH 750 analyzers. Stata /IC version 11 was used for statistical analysis. Mean pre-dialysis Hb was 102 g/L. Standard deviation (SD) of Hb was determined for each patient. The distribution of HbSD had a mode at 7 g/L and the upper quintile was 15-30g/L. Graphic analysis of Hb versus time for the upper quintile of HbSD depicted unexpected Hb variation and this was confirmed by chart review. Hb variation in hemodialysis patients could be attributed to clinical causes (anemia, acute bleeds or transfusions, dialysis pump-hemolysis), lab causes (analytic variation), or pre-analytic causes (blood dilution, or hemolysis). Analytic records revealed stable and precise measurement of Hb. Pre-analytic susceptibility of specimens to dilution, hemolysis or influence of tube-type was evaluated. Low-vacuum EDTA tubes were found associated with hemolysis due to inappropriate collection techniques. Collection tubes were replaced and staff educated about causes of hemolysis. A year of re-evaluation of variation in Hb is underway.

S089

Development of a Mobile app for Achieving Academic Excellence and Skill Development in Clinical Biochemistry

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Development of apps on mobile handsets as tools for personal and professional work has become a modern day necessity. This concept is also applicable to Clinical Biochemistry professionals involved in teaching, laboratory diagnostic services, research and developmental activities. One can make a humble beginning of developing an app for inter and intra communication between teacher, student and laboratory professionals. Subsequently, the contents in the app can be expanded to cover the syllabus of an academic course, lectures to cover the course, experiments thereupon, examination systems, evaluation procedures, self assessment and so on. With respect to skill development, downloading a video of specific contents from a you tube web site and practicing the skill, is the best option. Screen shots of such an app developed, which is relevant to clinical biochemists, will be shown and utility of this as a tool will be emphasized and discussed.

S090

Automated Serum Indices Assessment and Its Use in Reducing Laboratory Errors

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In the clinical laboratory setting, sample interferences viz., hemolysis, icterus and turbidity HIT), can be a significant source of laboratory errors with potential to cause serious harm for the patient. The prevention of errors is a major goal in healthcare. Toward this widely shared goal, laboratory errors have received a great deal of attention due to their impact on the quality and efficacy of laboratory performances and, in turn, on patient safety. So there is a need to systematically detect and reliably quantify the interferences (HIT) in every collected sample by means of objective and consistent technical tools that assess sample integrity. This is currently done by automated estimation of HIT index, available on almost all high end clinical chemistry platforms, like VITROS 4600, making the HIT detection reliable and thus helps in reporting patient test results more accurate. The objective of this study is to assess the frequency of samples having HIT interferences, as detected by the Microsensor technology in VITROS 4600 Chemistry system at clinical chemistry laboratory of Breach Candy Hospital, Mumbai and how it helps in reducing laboratory errors. In VITROS 4600 integrated system, microsensor technology is used to perform fiber-optic wave length scan of all the samples, to detect HIT indices and flag the results that are affected by the interferences. The number of samples having HIT indices above the normal range was recorded for a period of 30 days and analyzed, the results which are affected by these interferences. The frequency of samples showing HIT interferences are calculated and recorded. Out of 6825 samples analyzed, 15.36% samples showed hemolysis ranging from mild to gross, 3.26% of samples showed icterus and 0.39% of samples showed lipemia. In VITROS 4600 chemistry system, it has the facility to minimize the interferences due to HIT with the multi-layer microslide technology. But grossly hemolysed samples (0.22%) and grossly icteric samples (0.06%) showed the impact on few chemistries like K, Glucose, Cholesterol, Magnesium, total protein and albumin, etc. and it was flagged by the VITROS 4600 system automatically. So care was taken before releasing the report on such chemistries. The harmonization of HIT index of the samples processed, there is threshold limit for result acceptance for those

samples and flagging of the affected results in VITROS 4600. This helps in management of unsuitable samples and preventing the error in clinical chemistry reports released by the laboratory. This helps in improving the quality of the patient report with accuracy.

S091

Recent Advances in Diagnostics: Utility of Urine Metabolite Testing in Health & Diseases

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Metabolic profiling which mainly includes organic acid testing in urine specimens was traditionally used for several decades for diagnosis of inborn errors of metabolism. As the test can reveal an individual's metabolic strengths and weaknesses, the scope of organic acid testing has been markedly widened recently such that it can monitor physiological changes in nongenetic diseases and offer tremendous help in diagnosis and treatment of most chronic illnesses. The organic acids that are formed as intermediates in the process of metabolism are normally absent from urine or present at very low concentrations. When specific reactions in the metabolic pathways are blocked due to the insufficient enzyme or cofactor, the intermediates that precede the blocked step accumulate and spill into urine. New applications of metabolite testing include detection of metabolite markers in disorders of neurotransmitter metabolism and psychiatric disorders, mitochondrial disease and dysfunction, nutrient deficiencies, dysbiosis, autism, exposure to a wide variety of toxic chemicals from the environment etc. With Gas Chromatography Mass Spectrometry (GC/MS), organic acids/ metabolites are chromatographically separated on the basis of their polarity and volatility and then bombarded by an electron beam that fragments the eluting molecules in a characteristic pattern. The metabolites are identified from the characteristic spectral pattern. The concentrations of metabolites can be measured at levels of micromoles/ mole creatinine. Instead of tests that measure nutrient concentrations, abnormal concentrations of organic acids/ metabolites in urine provide functional markers of deranged metabolism, the root cause of most chronic diseases.

S092

Mass Spectrometry in Screening and Diagnosis of Inborn Errors of Metabolism

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Inborn errors of metabolism (IEMs) though individually rare, collectively occur with a frequency of 1:1500 people. The inherited enzyme defect results in disruption of specific metabolic pathways. The diagnostic target here is to either detect presence or absence of a substrate or product, deficiency of the enzyme or mutation in the corresponding gene. Single or tandem mass spectrometers (MS/MS, TMS) are used either directly or precluded with liquid or gas chromatography systems. Based on the target metabolites the samples used may be dried blood spot filter papers, urine filter papers, whole blood, urine, CSF, amniotic fluid and others. The applications range from new born screening or diagnosis of IEMs by assaying single or a panel of metabolites; or enzyme assays. The widely used panels include acyl carnitines, amino acids, organic acids and very long chain fatty acids. Based on the physicochemical properties the metabolites are ionized and fragmented in a reproducible manner. The ion intensity of each mass fragment is recorded by the detector and the mass spectrum is matched with the database libraries. For quantification intensity of a peak is assessed with its internal & external standards. In case of panel assays it may also be ideal to assess the ratios of the related metabolites in a pathway rather than an absolute quantification. The quality assurance measures ensure analytical sensitivity & selectivity with little or no interference while the clinical sensitivity and specificity measures should distinguish one disease from the other. Therefore, being a versatile technique with multiple applications, mass spectrometry is a robust technique widely used for new born screening and diagnosis of IEMs.

S093

LCMS_MS-Next Generation Revolutionary Diagnostic tool for Clinical Application with Special focus on Immunosuppresants

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In the clinical Biochemistry laboratories the advances has been revolutionary but not revolutionary in nature. A revolutionary step is the introduction of LCMS/MS into clinical laboratories. LC–MS/MS is an increasingly important tool in therapeutic drug monitoring & NBS as it offers increased sensitivity and specificity compared to other methods, and may be the only viable method for quantifying drugs without natural chromophores or fluorophores. The main attraction of HPLC-MS is high selectivity and sensitivity because this technique allows the quantification of the main drug independently of its metabolites. Very frequently, the immunosuppressive agents are used in combined regimens; in these cases HPLC-ESI- mass spectrometry is the best option for simultaneous analysis of several compounds in one short run.This cutting edge technology enables simultaneous quantification of various drugs. After performing an extensive validation, it is routinely been used at our centre of excellence for Therapeutic drug monitoring of immunosuppressant drugs. Thus Simultaneous

measurement of immunosuppressant drugs like Tacrolimus, Sirolimus, cyclosporine and everolimus can be done in single analysis. As compared to immunoassay techniques it's more specific as it does not have any cross reactivity with other analytes.The narrow therapeutic index, combined with the lack of surrogate markers of toxicity, adds to the empiricism in the administration of cancer therapy. The pharmacokinetics is highly variable in between patients. Taking advantage of TDM to optimize its usage in organ transplantation, LCMS/MS plays an important role. The choice of sample preparation method, column technology, internal standard and mass spectrometric conditions is important to ensure accurate drug measurement and to avoid interference from matrix effects and drug metabolites. Thus, LC-MS/MS is an attractive and versatile technique that facilitates rapid development of analytical methods, including new immunosuppressant's as they become approved for clinical use. Pharmacokinetics:LCMS/MS is commonly used in pharmacokinetics studies of pharmaceuticals and is the thus the most frequently used technique in the field of bio-analysis. These studies give information about how quickly a drug will be cleared from the body. MS provide high sensitivity and exceptional specificity. Proteomics/Metabolomics:LCMS/MS is also used in the study of proteomics where peptide mass fingerprinting to derive sequence of individual peptides. Drug Development:LCMS/MS is frequently used in drug development at different stages such as quantitative Bio-analysis, quality Control, Impurity Identification, In Vivo Screening etc.

S094

Different Mass Spectrometry based Technologies Used in Diagnostics

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Mass spectrometry is a powerful analytical technique used to quantify known & unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules. The complete process involves the conversion of the sample which may be solid, liquid, or gas, is ionized, by bombarding it with electrons. This may cause some of the sample's molecules to break into charged fragments which are then characterized by their mass to charge ratios.There are several types of mass spectrometry techniques, used in combination with chromatographic techniques for separation of ions. Gas chromatography technology is used for the identification and quantification of relatively small molecules (molecular mass <1,000 Da). Such molecules can be highly informative in newborn screening, toxicological and forensic applications, for delineating inborn errors of metabolism. The development and application of inductively coupled plasma mass spectrometry is widely used medical and forensic field, specifically, toxicology. Coupling of mass spectrometers to liquid chromatography further expanded the discriminatory power of the method and has important applications in Pharmacokinetics, Proteomics & Drug development. A resurgence of this technology is seen for studying larger molecules such as nucleic acids and proteins, due to the development of novel methodologies such as tandem mass spectrometry and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDITOF- MS). This a technical revolution allows easier and faster diagnosis of human pathogens than conventional phenotypic and molecular identification methods, is increasingly used in diagnostic laboratories. Thus Mass spectrometry is been used as a diagnostic tool in clinical laboratories for many decades and it has influenced many areas of diagnosis.

S095

What is Quality and Why Does it Matter?

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All providers of laboratory medicine services aim to provide a 'high quality' service but our understanding of what this means is variable. The IFCC has developed a 'quality ladder' to illustrate the stages of quality improvement. The aim of all laboratory specialists should be continuous quality improvement, effectively climbing the rungs of the quality ladder. The top of the ladder is reached through laboratory accreditation against the international standard ISO 15189:2012. Continuous quality improvement requires an active programme of quality management. This involves a continuous series of internal audits against locally established quality standards supplemented by benchmarking against external standards of performance. Documentation and document control are essential internal elements of quality management. External standards include: staff training and qualifications; performance in external quality assurance programmes; user satisfaction surveys; and, ultimately, full accreditation by external assessors against ISO 15189:2012. Everyone working in a clinical laboratory should contribute to continuous quality improvement.The results from laboratory medicine investigations inform a high percentage of all clinical decisions. Therefore, provision of a high quality service is of critical importance to patients, both in terms of patient safety and improving quality outcomes. In an increasingly litigious world avoidable quality failures in the provision of laboratory medicine services are unacceptable to patients and the public. Laboratory directors are being asked to provide evidence of their quality standards of performance. Contracts for service provision, clinical trials and research programmes are becoming conditional on provision of that evidence.

S096

ISO 15189:2012 A New Global Standard for Quality in Laboratory Medicine

Elizabeth Frank

The ISO 15189 standard was developed to bring globally consistent standards to the quality management system requirements for medical laboratories. The standard is being increasingly recognized worldwide as containing important new quality provisions that, when followed, can minimize the risk of compromising error, patient outcomes resulting from insufficient quality management and governance. The latest revision, ISO 15189:2012, introduces specific language concerning the quality management and performance monitoring of all the lab activity The ISO 15189:2012 standard helps medical labs develop their management systems and assess their own competence; it is also used by customers, regulatory agencies, and accreditation bodies to confirm or recognize the competence of a medical laboratory. This talk will give you an overview of the standards and how it can help you to achieve operational excellence

S097

Immune Response to Immunogen, Mitogen & Carcinogen: A correlation to LDLR Expression and Nuclear Cholesterol

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Environmental carcinogens are foreign bodies. Do they act as immunogen or behave differently? It is known that all carcinogens are mitogens and immune system cannot resist them. Objective was to explore the differential role of immunogen, mitogen and carcinogen on immune cells.Tetanus Toxoid (TT), Poke weed mitogen (PWM), Benzo alpha pyrene (BaP) and respective antibodies against LDLR, SREBP2 & cytokines. IgG estimation kit. Female Swiss Albino mice, RPMI 1640 and FCS. Treatment of animals, isolation of lymphocytes, Flowcytometric analysis, western blot, ELISA, ultracentrifugation, cholesterol estimation and statistical analysis. Three inducers viz. Tetanus Toxoid (TT), Poke weed mitogen (PWM) and Benzo alpha pyrene (BaP) popularly known as antigen, mitogen and carcinogen, have been explored in this study. It is apparent that all the three components have mitogenic properties but, they do differ in their immunogenic response. Being a typical immunogen, TT showed maximum immune response. Both PWM and lower concentration of BaP showed moderate immune response. At considerably higher concentration BaP makes immune cell death and thus facilitates its stay in the system. The critical concentration at which BaP paralyses the immune response was found matched with its reported carcinogenic dose.The findings in our study showed an impact of carcinogenic dose of BaP on T and B cell activity or in other words on immune response system.

S098

Prostate Markers

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Prostate cancer (PCA) is a major health concern across the world. Approximately Thirty years ago the diagnosis and management of PCA was revolutionized with the introduction of prostate specific antigen (PSA). However, recently, concerns have arisen regarding the inherent shortcomings of this biomarker and, as such, alternatives have been actively sought. Over the past decade new PCA biomarkers have been identified in tissue, blood, urine, and other body fluids that offer improved specificity and supplement our knowledge of disease progression. This presentation will focus on circulating biomarkers like-2 pro PSA, PCA3, IL-6, and TMPRSS2-ERG which are now commercially available kits and mention others, such as, microRNAs (miR-21, -221, -141) and exosomes which hold potential to become available as multiplexed assays.

S099

Wheatgrass and Colorectal Cancer

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In countries with a westernized lifestyle, about a quarter of deaths are cause by cancer, so it is an important problem and draws attention worldwide. Colon cancer is one of the leading causes of cancer related deaths in both men and women in western countries, including United States. Within Asia, the incidence rates of colorectal cancer (CRC) vary widely and are uniformly low in all south Asian countries and high in all developed Asian countries. The burden of CRC has risen rapidly in some economically developed Asian countries like Japan, South Korea and Singapore. CRC is one of the most common forms of gastrointestinal cancer and it is fourth most common cancer after lung, prostate and breast cancer. Diet is an especially attractive forum to modulate the inflammatory process. Dietary factors have been shown to modulate the pathogenesis of a variety of conditions, including celiac disease, colon cancer, and diverticulosis. Diet and nutrition clearly play a major role in etiology of colon cancer. Evidence from

epidemiological studies suggest that diet rich in fruits and vegetables are protective against colorectal cancer. The cereal grasses – wheatgrass, barley grass, alfa-alfa have been known to boost health and vitality both in humans and animals. The components of wheatgrass juice include chlorophyll; vitamins A, C and E; and various amino acids. It has been demonstrated that wheatgrass juice is anti-mutagenic. One constituent of wheatgrass is apigenin, which is believed to possess anti-inflammatory and antioxidant properties. Hence, the chemopreventive role of wheatgrass in CRC will be presented.

S100

Cripto 1: A Novel Tumor Marker for Oral Squamous Cell Carcimona(OSCC)

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Oral Squamous Cell Carcinoma (OSCC) is one of the commonest cancers in India. A high rate of mortality reported due to OSCC is majorly attributed to late diagnosis of the disease mainly due to non-availability of a screening tool or tumor marker. Cripto1 (CR-1), a member of the EGF-CFC protein family1 differentially expresses during early embryogenesis. Expression of CR-1 mRNA and/or immune-reactive protein, a key phenomenon in tumor dedifferentiation cancers, is associated with increased number of cancer stem cells, thus makes CR1 a potential target for a prospective tumor marker. In this we elucidated the potential role of Human Cripto 1 as a tumor marker in the cases of OSCC.31 biopsy proven OSCC cases and 30 age/sex-matched controls were recruited for the study. Serum CR1 level of controls as well as serum CR1 levels of the cases before and after standard therapy according to the stage of the disease were estimated by ELISA(R&D Systems^{TM}). Expression of CR1 was also checked at transcriptional mRNA level by Real time RT PCR and at protein level by IHC (Immuno-Histo Chemistry) in the cancer tissue. The data were analyzed by appropriate statistical tests for significance.There is significant (P=0.04) raise in the serum CR1 level in OSCC patients (mean 727pg/ml) with respect to controls (332pg/ml), which is significantly reduced (P=0.03) after completion of therapy in 100% cases. There is 4.32-fold increase in the mRNA expression of CR1 in cancer tissue with respect to the cancer free tissue and 68% of the cases showed 3+ cytoplasmic positivity for CR1 in tissue level in IHC. Human Serum Cripto 1 is a potential tumor marker for Oral Squamous Cell Carcinoma.

S101

DNA Methylation Alterations and POTE Gene Activation in Ovarian Cancer

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POTE is consisting of 13 autosomal and pericentromeric cancer germlineantigen genes, whose members are expressed in epithelial ovarian cancer (EOC) and solid tumor malignancies. DNA methylation changes are common in EOC, and frequently consist ofloss of DNA methylation genome-wide at specific repetitive DNA elements, including *LINE1*, and at pericentromeric regions. Determine whether DNA methylation changes or other epigenetic mechanisms regulate POTE expression and to define the potential functional contribution of POTE expressions in ovarian cancer. Affymetrix HG 1.0ST microarrays and RT-qPCR were used to determine *POTE* gene expression in normal ovary and EOC tumors. DNA methylation status of *POTE* and neighboring pericentromeric regions, where POTE genes reside, was determined by using sodium bisulfite pyrosequencing. Cell proliferation, cell migration, and cell invasion were determined following POTE knockdown or overexpression. POTE expression levels were highly elevated in EOC tumors showing global hypomethylation of *LINE1* elements, as compared to EOC tumors with hypermethylation of *LINE1* elements, linking global DNA methylation status to *POTE* expression. Methylation of pericentromeric DNA, as determined using *NBL2* pyrosequencing, was also closely linked to *POTE* expression and methylation status. POTE gene knockdown in EOC cell lines had minimal impact on cell proliferation and apoptosis, but resulted in significantly reduced cell migration and cell invasion. POTE hypomethylation and expression correlate with poor prognosis in EOC. These data establish a specific oncogenic role for DNA hypomethylation in EOC, and reveal POTEs as biomarkers and therapeutic targets in this malignancy.

S102

Involvement of MAP Kinase and *TSHR* pathway gladiators in the etiopathogenesis of Thyroid cancer

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Thyroid cancer is the most common malignancy of endocrine system. Recent molecular studies have described a number of abnormalities particularly in *RAS/RAF/RET* associated pathway that leads to the progression and dedifferentiation of thyroid carcinoma. Thyroid cancer sites among top 10 cancers in Kashmir (North India) and its frequency has doubled in a decade here. So, the main aim of the study was to elucidate the involvement of *RAS* and *BRAF* genes in association with silencing of *TSHR* gene in thyroid cancer. Mutational analysis of *RAS* and *BRAF* gene was performed by polymerase chain reaction (PCR) followed by DNA sequencing while as protein expression of *BRAF* gene was done by western blotting. Methylation specific PCR (MS-PCR) was set up for detecting promoter hypermethylation of *TSHR* gene. Overall mutations in exon 15 of *BRAF* identified in this study aggregated to 25% and all of them were affecting codon 600 and mutations were restricted only to papillary thyroid cancer (PTC). No mutations were observed in any of the three exons (*HRAS, NRAS* and *KRAS*). 90% of thyroid cancer cases showed increased expression of *BRAF*. There was a significant association between *BRAF* mutation and *TSHR* promoter hypermethylation in thyroid cancer patients ($P<0.05$). We conclude that both mutational events as well as over expression of *BRAF* gene are highly implicated in pathogenesis of thyroid cancer. Further a significant association was observed between the *BRAF* gene mutations and silencing of *TSHR* gene in thyroid cancer patients of Kashmir valley.

S103

A Study of Determination of Waterhardness and Prevalence of Hypomagnesaemia and Hypocalcaemia in Healthy Subjects of Surat District, Gujarat

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Various sources of drinking water are used with variable levels of total hardness, calcium and magnesium concentration(s). Inadvertent use of water purifiers can compound the problem of maintenance of desired levels of hardness. Studies of detection of hardness of different sources of drinking water and serum magnesium and calcium in normal subjects of Surat have not been carried out so far. Objective was to assess the concentration(s) of calcium, magnesium and total hardness in filtered and non-filtered water and its relation with serum magnesium and calcium in normal subjects consuming such water.Water samples both from rural and urban areas of Surat were analyzed for total hardness and calcium by complexometric and EDTA method respectively. Magnesium was obtained by deduction of calcium from total hardness. Serum samples of healthy individuals were analyzed for magnesium and calcium. Results were expressed in Mean±SD. Mean total hardness, calcium and magnesiumconcentration(s) in rural non-filtered tubewell water were much higher than the filtered water from the same area and magnesium concentration was significantly higher ($P=0.038$). Similar pattern was observed in urban municipal water and bottled water samples. Serum magnesium was significantly lower in population who were consuming filtered water compared to that of non-filtered water ($P<0.05$). No such difference was observed in serum calcium. Hypomagnesaemia is correlated with lower magnesium concentration in drinking water (both rural tubewell and urban municipal), which is attributed to use of water purifiers. Bottled commercial water was too soft with inadequate mineral content and hardness.

S104

Effect of Minor Changes in Dietary Habits by Consumption of Small Amounts of Raw Vegetables on Lipid Profile

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A good dietary regime is important for health. But to follow a particular regime is hurricane task. It is known from previous studies that there is an association between vegetarian diet and low total serum cholesterol as well as LDL-cholesterol which is a pointer to low risk of cardiovascular disease. Fruits and vegetables being rich sources of fiber and antioxidants have been the focus in our interventional study. Our current study focused on the difficulty of a change in the dietary habits; hence with a minor change in the daily diet for 90 days, we assessed the effect on the lipid profile and coronary risk factor. 20 Teaching and Non teaching faculty members of our college were included in the study. The age group was between 25 and 60 yrs of age with no metabolic disorders. Their Physiological and Biochemical parameters were measured. Biochemical parameter included Lipid profile and Blood sugar before the intervention. A slight modification in the diet was suggested with addition of raw vegetables and sprouts for a period of 90 days. Same parameters were then again measured. All the readings in mg/dl were Total cholesterol pre and post were 210.2

±37.7 and 203.8± 32.3. HDL Cholesterol pre and post were 45.2 ± 6.1 and 44 ±6.7. LDL cholesterol pre and post were132.5 ± 25.7 and 132.1 ± 24.3. VLDL Cholesterol pre and post were 32.2 ±20.6 and 27.1± 10.5. TG pre and post were 162.6 ± 102.7 and 135.5 ± 52.2. The CRI was 4.69 ± 0.8 and 4.66 ±0 .6. The results were not statistically significant but were decreased. Even a minor change in the diet can definitely have a good effect in lowering the lipid profile.

S105

Status of Haematopoietic Micronutrients (Vitamin B12 and Folic acid) in healthy population in a rural medical college in North-Western part of India

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In a recent study carried out in this Institution, vitamin B12 deficiency has been observed in general population inhabiting high altitude region of Kinnaur and Sub-Himalyan ranges of North-Western part of India. It was considered important to study the status of this vitamin and other haematopoeitic micronutrient status in a well to do population in a Medical College in this part of India. Objective was to study the status of haematopoietic micronutrients in the employees and Students (from economically well to do families) of DR RP Govt. Medical college, Kangra at Tanda in order to have an idea of overall status of vitamin B12 people in this part of the country.153 study units were picked by stratified random sampling method from the frame that comprised of all the students and employees of Dr. R.P. Govt.Medical College, Kangra at Tanda (both male and female), which were included in the study. The vitamin B12, folic acid, Homocysteine (Hcy) and Methyl malonic acid (MMA) levels were measured by chemiluminescent immunometric assay method using Immulite-1000. The present study revealed that ½ of the population (53.6%) of the volunteers had suboptimal B12 level (200pg/ml taken as threshold value) whereas the value of folic acid did not change significantly. The % age of the younger population with suboptimal B12 level was significantly more ($P<0.05$) than that of older population. A simultaneous hyper-homocysteinemia (>12uM/L) was observed in 73.6% of the volunteers. The level of MMA was also elevated (>11.75ng/ml) in 66% of the total subjects studied. There was thus inverse correlation between seum B12 and MMA and Hcy serum levels. Hence low B12 with corresponding high levels of MMA and Hcy can be taken as signs of –ve B12 balance with reasonable certainty. However more studies are required to elucidate the etiological factors responsible for deficient and marginal serum levels of B12 in our otherwise healthy study population. This is also required because of the fact that homocysteine alone has now been recognized to be attributable risk factor for causation of coronary artery disease (CAD).This important observation of hyper-homocysteinemia in the presence of biochemical evidence for B12 deficiency warrants its recognition as a public health problem and must be addressed vigorously by all available means.

S106

Role of Tocotrienols in Health and Diseases as a Supplement: A Review

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Tocotrienols are members of the vitamin E family, an essential nutrient for the body, made up of 4 tocopherols (α, β, γ, δ) and 4 tocotrienols (α, β, γ, δ). The slight difference between tocotrienols and tocopherols lie in the unsaturated side chain having three double bonds in its farnesyl isoprenoid tail. Tocotrienols are natural compounds found in selected vegetable oils, wheat germ, barley, saw palmetto and certain types of nuts and grains. Palm oil and rice bran oil represent two major nutritional sources of natural tocotrienols. Tocotrienols play an important role for the maintenance of the health and also fruitful for curing so many diseases. During the last 8 years, tocotrienol research has gained substantial momentum. More than 75% of the entire PubMed literature on tocotrienols has been published on or after 2002. This represents a major swing in the overall direction of tocotrienol research. The objective of this review is to highlight the potential significance of tocotrienol in overall health and diseases. Tocotrienol has antioxidant, anti-inflammatory, antineurodegenerative, antimicrobial, anticancer, antihypercholesterolemic, antiangiogenic etc properties.

S107

Effect of Adjunctive Treatment with Oral Antioxidants on the Levels of Oxidative Stress and Antioxidants in Schizophrenic Patients

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Schizophrenia, is a common psychiatric illness associated with a broad range of neurodevelopment, structural and behavioural abnormalities, which may result from genetic, environmental and lifestyle factors. Experimentally, these factors have been found to

cause the cellular metabolic stress that often results in oxidative stress increasing cellular levels of reactive oxygen species (ROS), over the antioxidant capacity. The present study investigates the effect of oral antioxidant supplementation along with antipsychotic treatment in limiting oxidative damage in schizophrenia. 90 schizophrenic patients were divided equally into three groups. Group-1:Newly diagnosed patients, without any medication or supplementation. Group-2: Patients on antipsychotic treatment since three months Group-3: Patients with oral supplementation with antipsychotic treatment since three months. Control group had 30 healthy individuals. Level of oxidative stress and antioxidants (enzymes, vitamins and endogenous substances) was assessedin whole blood and plasma as per standard methods. Baseline psychiatric symptoms were accessed by Positive and Negative Syndrome Scale. Level of oxidative stress increased in Group 1 and Group 2 with a relative decrease in levels of antioxidants as compared to controls. In Group 3 oxidative stress reduced and level of antioxidants improved. A parallel improvement in the psychopathology indicated prevention of the oxidative damage contributing to the pathophysiology of schizophrenia. This study supports the augmentation of antipsychotic treatment by oral supplementation with a combination of antioxidants from the very onset of psychosis to reduce the oxidative injury and improve the clinical outcome of the illness.

S108

Association of Total Antioxidant Status (TAS) with Primary Open Angle Glaucoma Patients of North Indian Population

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Primary open angle glaucoma (POAG) is defined as a progressive chronic optic neuropathy which is characterized by the loss of optic nerve axons and the related retinal ganglion cells. It is the leading cause of irreversible blindness and the second most common cause of all blindness after cataracts. Estimation says that there were 60.5 million people with primary glaucoma in 2010 and there will be 79.6 million by 2020 and which will result blindness in 11.2 million people by 2020. One factor becoming more likely to be involved in pathogenesis of POAG is oxidative stress. Our study included 112 POAG cases (60 were males and 52 were females) and 110 controls (57 were males and 53 were females). Levels of Ferric reducing antioxidant power (FRAP) was determined by spectrophotometric methods. The mean ages were 49.88 ± 12.34 and 53.74 ± 11.87 years in POAG cases and control groups respectively. Total antioxidant status levels in plasma were 8.73±0.42 in cases and 13.8±0.60 in the controls. Total antioxidant status levels in plasma were significantly lower in cases as compared to controls (P=0.001). Findings of this study suggest that there is some correlation of oxidative parameters with POAG. Here we demonstrate that TAS level was decrease in POAG patients. If other studies produce sufficient specificity of TAS with progression, then this could be used as an easy detectable marker for POAG severity.

S109

To Assess the Nutritional Status Among Male and Females Diabetic Patients

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Diabetes mellitus is a group of disease characterized by high blood glucose concentration in the blood and alteration in carbohydrate, protein and fat metabolism. People are greater risk of diabetes due to improper dietary practice, unhealthy life style, lack of physical exercise. The present study was conducted a comparative study of nutritional status among diabetic males and females. Multistage stratified random sampling technique was used for selecting 100 samples in both male and females and an interviewed schedule was developed to collect information regarding socioeconomic profile, dietary pattern etc. Dietary intake between males and females diabetic were highly significant but age, BMI, etc. between males and females diabetic were insignificant. Consumption of high fat and carbohydrate diet was revealed as the major contributing cause of disease in both males and females.

S110

Biomarkers in Tuberculous Pleural Effusion

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Tuberculous pleural effusion is 30% of the TB cases in India (2 million). TB PF effusion shows abnormal protein, LDH and glucose. Diagnostic challenges led to newer tests like ADA & Interferon γ. ADA a non-specific inflammatory marker produced and released from macrophages, activated lymphocytes and neutrophils occurs as 2 isoenzymes ADA1&2. ADA2 released by activated monocytes and macrophages contributes mostly to the total ADA activity. ADA and ADA1/ADA ratio found to give a higher sensitivity and specificity. Interferon γ release assays are useful in detecting latent Tb infection. Aim of the present study wasto evaluate the role of ADA isoenzymes and Inteferon γ in tuberculous pleural effusion.154 Patients with exudative pleural effusion underwent PF aspiration & pleural biopsy. Protein, LDH,

ADA, IFN and cultures were done. ADA & ADA1 were measured enzymatically using substrates adenosine and deoxyadenosine. IFN γ by ELISA. Cut off values, sensitivity, specificity, PPV, NPV and AUC of ADA, ADA2, and IFN γ were obtained using ROC curve analysis. Combination of biomarkers, improved sensitivity but specificity declined. Tuberculous PF is one of the common causes for pleural effusion with a diagnostic challenge. The pleural fluid biomarkers, combination of tests and scoring system have an important role in diagnosing TB effusion but are not sufficient to replace the conventional gold standard cultures for diagnosis.

S111

Altered Ferroxidase Activity of Ceruloplasmin in Chronic Obstructive Pulmonary Disease

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Oxidative stress is one of the major patho-physiologic hallmarks in the development of COPD. Ceruloplasmin, the major serum inhibitor of lipid peroxidation has been documented as a main extracellular antioxidant in serum and play a role in preventing lung injury, and abnormality in its oxidative inhibition could be involved in pathogenesis of COPD. This study aims to explore the correlation between ceruloplasmin and its ferroxidase activity in COPD. Ferroxidase activity of ceruloplasmin was estimated by kinetic, enzymatic Somani-Ambade method, while ceruloplasmin was estimated by immunotubidimetric method using commercially available kit. Serum ceruloplasmin and ferroxidase activity were significantly higher in COPD patients as compared to normal controls. Mean ± SD in COPD versus controls respectively are ceruloplasmin: 45.84 ± 12.7 mg/dL versus 37 ± 9.7 mg/dL; ferroxidase: 1324.9 ± 278.53 IU/L versus 980.5 ± 202.3 IU/L. Statistically significant & good correlation was found between ceruloplasmin and ferroxidase in controls, nonsmoker controls and smokers controls (r = 0.76, 0.71 and 0.79 respectively) while in COPD, COPD nonsmokers and COPD smokers, no correlation was found (r = 0.00, 0.29 and 0.09 respectively).There is alteration in the ferroxidase activity which may be due to any reason like modification in the structure of ceruloplasmin due to the oxidative stress in COPD.

S112

Prenatal Screening for Fetal Aneuploidy

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New developments in maternal serum and ultrasound screening have made it possible to assess the risk of having a fetus with aneuploidy (trisomy 13, 18, 21) by a non-invasive screening test and to determine whether invasive prenatal diagnostic testing is necessary. Invasive prenatal diagnosis would be advised only to women who screen above a set risk cut-off level. Objective was to find risk of double (first trimester screening), triple and Quadrupal marker (second trimester screening) in pregnant females and also study their outcome. 188 pregnant females visiting Gynae OPD of tertiary care hospital were screened for Dual marker (83 females), Triple and Quadruple marker (19 and 86 females respectively). Serum free β HCG and PAPP-A was estimated for Dual marker. If risk observed, it was followed by second trimester screening. For triple marker, AFP, β HCG, Unconjugated estriol were estimated. In Quadruple marker, Inhibin A was included to triple marker tests. AFP, β HCG and PAPP-A analyzed on Elecsys (e 411) Roche. Unconjugated Estriol and Inhibin A were analysed on Access 2 - Beckman coulter. Risk was estimated using SSDW (Software &soft for Down syndrome) from Roche. Females with high risk were further investigated by amniocentesis. Risk was observed in 19 females, 2 (2.4 %) for Dual marker, 4 (21%) for triple marker and 13(15%) for quadruple marker. Six females did not report for follow up, another seven refused amniocentesis. Six patients got the amniocentesis done; only one patient had positive risk. Six patients had ineviatble abortion, remaining seven are continuing pregnancy and results are still awaited.Only 10% females were advised amniocentesis. Non- invasive biochemical screening is cost effective and has the benefit of reducing the numbers of normal pregnancies lost because of complications of invasive procedures.

S113

Biomarkers in The Management of Cardiac Emergencies

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Heart failure is a complex clinical syndrome in which the pumping function of the heart becomes insufficient [ventricular dysfunction] to meets the needs of the vital systems & tissues of the body. HF [heart failure] is progressive & chronic

disease, worsening every time. HF is caused by progressive remodeling of the heart, a process that changes its size & shape & subsequently impairs the function of the ventricles. Systolic HF thinning & weakening of the ventricle walls results in dilation & reduced capacity to eject blood [reduced ejection fraction]. Diastolic HF thickening & stiffening of the ventricles due to hypertrophy results in impairs relaxation [preserved ejection fraction]. Any structural or functional disorder that leads to deterioration of heart muscle function may lead to HF. Diagnosis of Acute heart failure is difficult because there are many varied & often nonspecific, clinical symptoms [dyspnea, chest pain, fatigue, and cough]. NT-pro BNP is highly sensitive & specific for the diagnosis or exclusion of acute HF & it is powerful & cost effective adjunctive tool for clinician in the diagnosis & the medical decision-making process [risk stratification & therapy selection. Whole blood [EDTA] sample is required.Test is based on immunoflourance technique. Test is done on ELISA reader, Antibodies coated with pro hormone fragment like pro NP77-108 [C-Terminal 32 amino acids for BNP & pro NP 1-76 [N- terminal 76 amino acids for NT-Pro BNP are requried & their half-life for BNP is 20 minutes &NT pro BNP 60—120 minutes. NTpro BNP is highly sensitive & specific for the diagnosis or exclusion of acute HF. If NT proBNP <300pg/ml - HF unlikely further evaluation of non–cardiac cause of dyspnea, if NTproBNP> 450pg/ml -HF likely this value is also varies with age. Diagnosis of acute heart failure is difficult because there many varied & often non-specific clinical symptoms [dyspnea, chest pain, fatigue, cough]. The diagnosis is based on the combined use of patient history & physical examination, ECG, chest X-ray, echocardiography & laboratory tests, including natriuretic peptides act as important biomarker of HF. Suspected heart failure [Assess signs & symptoms] - Known heart disease, chronic HF, Abnormal ECG, Congestion on X-ray, Abnormal blood gas, Elevated natriuretic peptides. Brain natriuretic peptide [BNP] is a cardiac hormone primarily synthesized in & secreted from the myocardial ventricles. This hormone has been shown as a sensitive & specific marker for changes in ventricular function;it appears in blood after the cleavage molecule Pro NP. This cleavage also results in the release of NTpro BNP the N-terminal counterpart, Therefore the blood levels of both BNP & NTproBNP are increased in heart failure but BNP only increased in HF & NTproBNP is highly sensitive & specific for the diagnosis or exclusion of acute HF it is powerful & cost-effective tool for clinician in the diagnosis & triage of patients with acute dyspnea. Circulating concentration of BNP is increased in individuals with chronic heart failure & appears to correlate with its severity. NTproBNP has utility as both a rule-out & rule–in test for heart failure.

S114

Serum Procalcitonin as a Biochemical Marker for Surgical Site Infection

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Surgical site infection (SSI) is a diagnostic challenge particularly in surgeries requiring application of prosthetic implants. Patients present with sero-sanginous wound discharge at the time of first post-operative wound dressing, which may turnout to be precursor for frank SSI. Wound debridement if done early in such cases can be very fruitful in controlling the infection. We thus aimed to study the role of procalcitonin in predicting SSI in patients undergoing clean elective orthopaedic procedures. 116 patients with no infective foci, undergoing clean elective orthopaedic operations were included in the study. Serum samples were drawn just prior to surgery, on post op day-1 and post –op day3. The levels of procalcitonin were evaluated in these serum samples by using commercially available ELISA Kit. 116 patients were divided into three groups – no wound complication (87), culture negative wound discharge(19), and culture positive wound discharge(10). The serum procalcitonin levels were increased (P value<0.001) in patients having postoperative wound discharge. On comparing the levels in patients with culture positive v/s culture negative wound discharge there was a statistically significant increase in the levels of procalcitonin on post-op day 3 (P value <0.001). It was further derived that serum procalcitonin level of 70.23pg/ml or greater on post-op day 3 could predict the presence of surgical site infection with an accuracy of 97.4%. Estimation of Serum Procalcitonin levels in the post-operative period can guide the surgeon to perform an early wound lavage and debridement in such cases.

S115

IMA Levels in Cord Blood Plasma in Pre-eclampsia

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Pre-eclampsia is associated with ischemia and increased oxidative stress in maternal circulation extending to neonates also. This effect may lead to modification of plasma albumin to ischemia modified albumin (IMA). The effect of pre-eclampsia on plasma levels of IMA was assessed in cord blood samples and compared with healthy neonates. IMA levels were estimated in cord blood of thirty newborns born to pre-eclamptic mothers and

compared with thirty normal newborns. IMA was estimated colorimetrically and the results were compared statistically. The levels of IMA were found to be significantly higher (P<0.001) in newborns born to pre-eclamptic mothers (0.835± 0.02 ABSU) as compared to those born to normal mothers (0.325± 0.01 ABSU). IMA may act as a marker of ischemia and oxidative stress in newborns delivered to pre-eclamptic mothers.

S116

Procalcitonin as early Biomarker for Bacterial Infection & Sepsis

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Sepsis is the systemic response to infection by microbial organisms. A differential diagnosis of infection caused by either bacteria or other microbial organismsis essential for effective treatment and prognostic assessment. During bacterial infection PCT is secreted in large quantities by body tissues, contribute to sepsis related death. In normal healthy people PCT levels are <0.05 ng/ml. Objective was to evaluate PCT as early biomarker for diagnosis of bacterial infection/sepsis. PCT levels were monitored for patients admitted with H/O fever and infection. Patients were grouped as(A<0.5), (B=0.5-<2.0), (C=2.0-<10.0), (D=10-<50.0), (E=50-<100), (F=100.0) as per PCT levels. PCT were analyzed on Cobas e411 on day of admission (T1) and followed with antibiotic treatment on T2 upto T10 times, for all the groups (with sepsis) except group A. Group A was subdivided into A1 (PCT<0.25) & A2 (PCT =0.25 - <0.5). Blood culture and PCT was repeated without any antibiotic treatment after 24 hrs for group A1 & A2. On T1 patients (n=2055) were grouped as (A-32% B-24% C-23% D-13%E-3.0% F-4.5%). Group A1 (n=299 mean 0.11) and group A2 (n=304 mean 0.329). On T2 group A1 (n=299 mean 0.108) and from A2 (n=246 mean 0.325) have shown in significant variation in PCT levels after 24 hrs. PCT value (n=59 mean 0.434) co-related with positive blood culture report. PCT values <0.25 indicate low probability for bacterial infection. PCT values >0.35 co-related with bacterial load and positive culture reports. PCT testing shown 90% accuracy for early diagnosis of bacterial infection.

S117

Hyperleptinemia and Leptin Receptor Gene Polymorphism in Type 2 Diabetic Subjects

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Leptin is a 16kDa protein synthesized by the white adipose tissue, which regulates the body weight and energy expenditure. Leptin has a role in cardiovascular homeostasis that are potentially atherogenic, thrombotic and angiogenic.The effect of adipocytokines like leptin has a link between insulin resistance and vascular dysfunction. The biologic activities of leptin on target tissues are carried out through selective binding to a specific receptor, *LEPR* (1p31 chromosome).The main objective is to estimate leptin levels and evaluate LEPR gene polymorphism (Q223R) in Type 2 Diabetic subjects and correlate with cardiovascular risk markers. The study included 40 Diabetic subjects with poor glycemic status attending the Diabetic clinic of SRM Medical College Hospital. Genotypic analysis of Q223R polymorphism was done with conventional PCR technique. Correlation was made with phenotypic variables using X^2 test. Serum Leptin was estimated using DRG-ELISA kit and other lipid profile parameters (TC, TGL, HDL-C, LDL-C) were also estimated. Mean serum Leptin levels was elevated in Type 2 Diabetic subjects and showed statistically positive correlation with waist circumference, TC/HDL-C, Non-HDL-C and HbA1C; and negatively correlated with HDL-C. Hyperleptinemia was confined statistically with Q223R LEPR gene polymorphism. LEPR gene polymorphism (Q223R) and hyperleptinemia in uncontrolled T2DM confers a link between insulin resistance and adverse cardiovascular outcome.

S118

Correlation of Lipid Profile and Uric Acid in Pre-eclampsia of Third Trimester

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Pre-eclampsia affects approximately 3% of all pregnancies worldwide, with onset of symptoms in the late second or third trimester, commonly after 32nd week. It is common in nulliparous women. To avoid complications it is necessary to diagnosis it in advance, but the available tools are unable to clinch the diagnosis of preeclampsia effectively in majority. Objective was to find out an association of lipid profile and uric acid with pre-eclampsia in

nullipara pregnant women in third trimester. One hundred nulliparous pregnant women in their third trimester of 18-35 years were divided into; fifty pre-eclamptics of study group and fifty non pre-eclamptic in control group; further subdivided according to age, 18-26 and 27-35 yrs. Diagnosis was confirmed as per the standard criteria. Lipid profile and uric acid levels were estimated by Vitros 250 dry chemistry analyzer. Data was analyzed statistically by student t test at P<0.01 level of significance. TC, LDL-C and VLDL-C levels in the study group as a whole and in the patients between 18-26 years were significant; HDL-C levels in the patients between 27-35 years were significant while TG and uric acid levels in all the three study groups were significant. Total cholesterol, LDL-C, VLDL-C, triglycerides and uric acid levels were raised in preeclampsia and statistically significant; while HDL-C levels were raised in these patients but statistically non-significant, it can be concluded that there exists an association in lipid profile and uric acid with pre-eclampsia therefore dyslipidemia and raised uric acid levels are the features of pre-eclampsia in nullipara pregnant women in their third trimester.

S119

Adiponectin: Role in Glucose Homeostasis in Relation to BMI in Type 2 Diabetics

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Amongst the many adipocytokines discovered in recent times, adiponectin has drawn much attention for its insulin sensitizing, anti-atherogenic and anti-inflammatory actions. Though adiponectin is secreted by adipose tissue, its decreased levels are associated with obesity and T2DM.The ever increasing incidence of obesity and type 2 diabetes mellitus is compelling us to look for newer approaches to understand the complex pathophysiology underlying these conditions and search for new therapeutic targets. In keeping with this, the present study was conducted to study the role of adiponectin in glucose homeostasis in T2D subjects in relation to BMI. Seventy five T2 DM patients visiting endocrinology OPD of a tertiary care hospital were selected for the study. Patients on thiazolidinediones were not included. They were divided into three groups of 25 each. Group A: < 25kg/m^2, Group B: 25-29.99kg/m^2, Group C: =30kg/m^2. Serum adiponectin levels were estimated by ELISA (Human ELISA kit), FBS, CRP, HbA1c, insulin and lipid profile was analysed on Cobas 6000 (Roche). Insulin resistance was measured by homeostasis model assessment (HOMA-IR). 56% patients had low adiponectin levels (<10 µg/ml), only 10.8 % patients had >25 µg/ml. Group B patients had lowest levels of adiponectin (11.86±8.79µg/ml). FBS, HbA1c, insulin levels and HOMA-IR showed a negative correlation with adiponectin in all the groups but was significant in group B only (r= -0.555, -0.611, -0.477and -0.528 respectively). CRP and all the fractions of lipid profile (except HDL) showed negative correlation with adiponectin in all the groups but it was not significant. HDL levels increased with increase in adiponectin levels in all the groups.This study reaffirms the significant role that adipose tissue hormones play in path physiology of T2DM. Significant negative correlation of glucose, HbA1c, insulin resistance with adiponectin noted in overweight diabetics supports the hypothesis that these patients can be potential therapeutic target for drugs increasing adiponectin levels.

S120

Evaluation of Serum Levels of Ischemia Modified Albumin and Albumin Adjusted-IMA in Patients with Diabetic Retinopathy and Healthy Controls

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Increased serum levels of ischemia modified albumin (IMA) are reported in diseases associated with ischemia and oxidative stress. Because of dependence of IMA values on albumin level, it would be important to report albumin levels and IMA values corrected for albumin interference. There is no published report about serum concentrations of both IMA and albumin adjusted-IMA (AAIMA) in patients with DRP in India. Objective was to evaluate serum levels of Ischemia modified albumin (IMA) and albumin adjusted-IMA (AAIMA) in patients with diabetic retinopathy (DRP) and healthy controls.This case control study was done on 18 DRP patients and 23 healthy controls. Serum was obtained to measure lipids, albumin and IMA. IMA level was measured by colorimetric albumin cobalt binding assay and values were presented as absorbance units (ABSU). IMA results obtained were adjusted for albumin interference and AAIMA was calculated by using a formula [Individual serum albumin/median albumin concentration of the population X IMA (ABSU)]. Serum IMA and AAIMA were significantly higher in patients with DRP compared to controls. (P<0.01and P<0.05, respectively), whereas albumin and HDL-Cholesterol were significantly lower in DRP patients (P<0.05 and P<0.01 respectively). This study is an evidence for involvement of oxidative stress damage in the pathogenesis of DRP, which is reflected as increased IMA and AAIMA values with decreased albumin and HDL-Cholesterol levels.

S121

Effect of Lipid Profile upon Prognosis in Ischemic and Haemorrhagic Cerebrovascular Stroke

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Stroke is the third major cause of death worldwide. Elevated plasma concentration of low density lipoproteins and low plasma concentration of high density lipoprotein concentration are associated with an increased risk of atherosclerosis and coronary heart disease but the relation between serum lipids, and cerebrovascular disease is less clear. The Aim of this study was to investigate the reliability and accuracy of serum lipid profile in assessing the prognosis/neurological worsening in patients with ischemic and hemorrhagic cerebrovascular stroke.The subjects in the present study comprised of 101 healthy controls and 150 cerebrovascular stroke patients (including 90 with ischemic stroke and 60 with intracerebral hemorrhagic stroke). For control subjects, early morning fasting blood sample was collected. For patients with stroke, the venous blood samples were drawn within 72 hrs from the onset of symptoms. The samples were processed as per the laboratory protocol, and the lipid profile parameters, were assayed on the same day, using the ERBA Chem 5 semiautoanalyser. The disability was assessed by National Institute of Health Stroke Scale at the time of admission (within 72 h from the onset of stroke) and on 7th day after admission. A statistically significant association was observed ($P< 0.001$) between the parameters of lipid profile of cases and healthy controls, and also with the prognosis of the stroke.This study points to the need to stratify cerebral vascular accidents based on their type (ischemic or hemorrhagic) in order to determine the real association between lipid alterations and occurrence of these neurological events. This study may also help guide future trials attempting to relate lipid alterations with occurrence of vascular events.

S122

Study of Holotranscobalamin and Vitamin B_{12} Levels in Patients Receiving Chemotherapy

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The load of malignancy in our country is bound to increase dramatically. Vitamin B_{12} commonly known as Cyanocobalamin is a water soluble vitamin which has a very important role in synthesis and maturation of genetic material. Its level in Indian patients receiving chemotherapy is not available and hence this study was done to determine its levels in cases with malignant diseases. Objective was to determine whether there is any effect of chemotherapy on the levels of the above vitamins in the blood and to predict whether there should be need of any vitamin supplementation accordingly.This study is a prospective study carried out on 50 cases of malignancies undergoing treatment in the Oncology Department of Command Hospital. Blood sample was collected before and after chemotherapy in the first 2 cycles of the treatment regimen. Serum holotranscobalamin & serum vitamin B_{12} (by ELISA)and serum homocysteine was measured colorimetrically.The value of serum holotranscobalamin before chemotherapy was 84.36 pmol/L, values while the subsequent values were between 97-106.8 pmol/L. The value of serum Vitamin B_{12} before chemotherapy was 578.4 pgm/ml, while the subsequent values were 451.1-600.03 pgm/ml. The value of serum Homocysteinein the samples were 9.96 -10.49 µmole/L. 15 patients with initial deficiency of holotranscobalamin had normal values subsequently. Thus there is a role of vitamin B_{12} supplementation in patients receiving chemotherapy.

S123

Characterization of Biomarker and Therapeutic Target for Triple Negative Breast Cancer

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The biology and the molecular mechanisms which drive the growth and metastasis of Her-2 negative breast cancer are not understood. Accumulating evidence indicates that selective overexpression and activation of epidermal growth factor receptor (EGFR) and Src regulates oncogenesis in this phenotype. Here we show that known substrate of Src, Annexin A2 (AnxA2) and its downstream autocrine factors like IL-6 and HB-EGF expression also increases with the progression of the Her-2 negative breast cancer. In addition to its known function in focal adhesion kinase and cytoskeletal rearrangement, here we report that AnxA2 in the membrane microdomain might be involved in maintaining the constitutive activation of EGFR downstream signaling intermediates and so also in cell proliferation, migration and viability. In cancer cells, the membrane bound AnxA2 has a major role in tumor invasion and metastasis. This glycosylated AnxA2 at the membrane surface is a good therapeutic target. We have purified a plant lectin, which has high affinity for membrane surface AnxA2. The treated lectin competes with other proteins for binding with AnxA2. This specific binding of lectin abrogates the downstream signaling activity of AnxA2 thereby decreasing the expression of antiapoptotic Bcl-2 family proteins and activates the apoptotic proteins. The functional relevance of lectin binding was confirmed by DNA fragmentation study and MTT assay in Triple Negative Breast Cancer cells. This illustrates that targeted lectin therapy can

be a novel strategy to target AnxA2/EGFR autocrine loop in triple negative breast cancer.

S124

Evaluation of Salivary and Serum Tumor Markers in Breast Cancer Patients

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Saliva is an important and necessary body fluid. In the last 10 years the use of saliva as a diagnostic fluid has become somewhat of a translational research story. Based on this, a study has been carried out in our department to evaluate the salivary and serum tumor marker level in breast cancer patients. Unstimulated saliva and blood samples were collected from the histopathologically diagnosed breast cancer patients from our OPD after getting institutional ethical clearance and before initiation of any treatment in fasting condition. The study group comprised of 100 patients and control group comprised of 25 healthy normal individuals in the same age group from our hospital staff and volunteers. Blood samples were centrifuged at 2000 rpm for 10 minutes. The collected saliva was also centrifuged within one hour to eliminate debris and cellular materials. All the marker (CEA, CA 15-3, and CA 125) levels in both samples were measured on the same day by using Vitros Eci (Ortho clinical Diagnostics Ltd) CLIA instrument as per manufacturer's procedures. The values were analyzed using SPSS statistical software and expressed as mean $\pm$ SD, the levels of significance were determined by Student's 't' test. In our study we found that there was a significant positive correlation between Salivary and serum concentration of these markers. Salivary analysis is advantageous due to the easy and non-invasive method of collection, safety and the possibility of repeated collection without discomfort to the patients. So tumor marker analysis of saliva can be used (an alternate to blood) to diagnose and monitoring treatment procedure and disease recurrence in breast cancer patients.

S125

A Tumor Marker with Clinical Information's used in Diagnostic Laboratory for Cancer Treatment or Screening

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The definition of a tumor marker has evolved considerably over the past two decades, principally as a result of advances in technology and redefinition by federal regulatory agencies. These markers have been used to supplement clinical findings and other diagnostic procedures, which hold great promise for management of patients care. So are the tumor markers permits as stand alone in the diagnosis for cancer patients? Although some heterogeneity occurs with each tumor marker and its associated malignancy, they provide clinically useful information when positive, and may be incorporated into management and monitoring of cancer patients. There are many instances where blood tumor antigens such as CA 15-3 and CEA are elevated in approximately 80% of patients when they present with symptomatic metastatic disease. Despite clinical indications for the diagnosis and monitoring of metastatic breast cancer, blood tumor antigens are of no value in screening and early detection of primary disease. A similar case like AFP and LDH in hepato-cellular carcinoma and AFP and beta-hCG intesticular cancer. Also, bone scans are probably not necessary in prostate cancer patients with PSA levels <20 ng/ml. The measurement of tumor markers in serum must be integrated with other technical advances in laboratory that relate to similar clinical questions. The important improvements and availability of new radiological imaging methods are also used in initial diagnosis and detection of recurrent or metastatic disease. The optimal use of markers should also ensure reduced costs, optimal patient outcomes as well as the ability to generate more data on emerging tumor markers so that progress is not impeded. So, tumor markers have not been useful in screening because of lack of specificity, but are a useful adjunct in predicting recurrence and assessing efficacy of treatment.

S126

Detection of Human Papilloma Virus DNA in Urine Specimens can be used as Non Invasive Mass Screening Tool for Preventing Cervical Cancer

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Cervical cancer is global public health problem and most common cancer in women in developing countries. Most cervical cancers are curable and can be prevented by regular screening . Invasive screening methods including PAP test , high risk human papilloma virus (HPV) detection in cervical smears along with gynecologic examination are being used to reduce morbidity and mortality of women by cervical cancer. This Study was carried out to evaluate the usefulness of human papilloma virus detection in the urine of women and establish a non invasive mass screening tool for preventing cervical cancer. 1000 urine and 1000 cervical samples from 1000 women were analyzed. Polymerase chain reaction (PCR) was performed on these 2000 samples using Biotools-Spain kit for high risk HPV detection. The concordance of overall results between both sample groups was 80%. Further,

in women with high-grade squamous intraepithelial lesion PCR detected HPV in both urine and cervical samples, here concordance between the two groups was 100%. Thus, HPV detection in urine samples may be used as a mass cervical cancer screening tool in women with poor gynecologic attention and invasive screening methods may be avoided. Especially, in developing countries where motivating women for invasive screening methods is very difficult. Financial grant for the study from DBT GOI is deeply acknowledged .

S127

A New Era in Prenatal Care-Noninvasive prenatal testing Using cell free fetal DNA for fetal Aneuploidy

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Non-Invasive Prenatal Diagnosis (NIPD) has been one of the most fascinating fields during the last decade and is rapidly evolving. Traditional non-invasive prenatal screening for autosomal aneuploidies involves screening via a combination of ultrasound and serial detection of maternal serum markers in the first and second trimesters, with follow-up diagnosis by invasive procedures such as amniocentesis or chorionic villus sampling (CVS). The discovery of fetal cfDNA in maternal circulation during pregnancy raised the possibility of new methods for non-invasive detection of fetal chromosomal abnormalities. Noninvasive prenatal testing uses cell free fetal DNA from the plasma of pregnant women and have a tremendous potential as a screening tool for fetal aneuploidy. The American College of Obstetricians and Gynecologists has recommended that women, regardless of maternal age, be offered prenatal assessment for aneuploidy either by screening or invasive prenatal diagnosis regardless of maternal age; cell free fetal DNA is one option that can be used as a primary screening test in women at increased risk of aneuploidy. Cell-free DNA fragments are short fragments of DNA found in the blood. During pregnancy, there are cell-free DNA fragments from both the mother and fetus in maternal circulation. It is possible to analyze cell-free DNA to detect common fetal trisomies such as Down syndrome (trisomy 21). Some of the methods used are the massively parallel genomic sequencing, which uses a highly sensitive assay to quantify millions of DNA fragments in biological samples as early as the 10th week of pregnancy and another is the chromosome selective sequencing which has a more targeted approach for the detection of aneuploidies.

S128

Indian Reference Intervals: C-RIDL IFCC Initiative

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The IFCC committee on reference interval and decision limits (C-RIDL) initiated a worldwide multicenter study on references values in 2011 facilitating the implementation of country specific reference intervals (RIs) by providing a common protocol. With limited literature on Indian population specific RIs, the present study aimed to derive RI for 49 routine biochemical analytes in healthy Indian population based on the C-RIDL protocol. In this cross-sectional priori study a total of 512 healthy Indian volunteers were recruited. Serum collected from an overnight fasting blood sample was analyzed for 49 routine biochemically and immunologically measured analytes. Multiple regression analysis (MRA) and 3-level nested analysis of variance (ANOVA) were used to identify the potential sources of variation of reference values. For statistical derivation of RI, parametric and nonparametric methods were used. Need for iterative optimization procedure called latent abnormal values exclusion (LAVE) method were also examined. MRA results indicated that both age and BMI were apparent sources of variation for many analytes in both males and females. ANOVA revealed that partition of RIs by gender and age was required for 22 (ALB, CRE, UA, TBil, TG, HDL, Fe, ALT, AST, GGT, CK, CRP, IgM, TRF, TTR, FERR, CEA, CA125, PRL, tPSA, fPSA and FT3) and 12 analytes (ALB, UN, GLU, TG, TChol, LDL, Ca, FERR, AFP, tPSA, fPSA & PRL) respectively. In majority of the analytes, RIs derived by parametric method were narrower than by nonparametric method, reflecting somewhat distorted peripheral distributions of their reference values. Application of LAVE method led to narrowed width of RIs for analytes affected by metabolic disorders.The present study has for the first time provided more comprehensive information on reference intervals, partition of reference values and source of variations for 49 target analytes in Indian healthy volunteers. The final RIs adopted were those derived by parametric methods with or without optimization by use of LAVE method depending on its efficacy.

S129

Comparison of Cockcroft Gault & MDRD Equations in Estimation of GFR in Chronic Kidney Disease

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GFR may be estimated by using Cockcroft Gault (CG) or MDRD formulae using routinely estimated analytes like creatinine, urea and albumin in serum. Objective of the present study was to ascertain the accuracy of estimation of GFR using Cockcroft Gault and MDRD formulae in patients of chronic kidney disease using Gates method as the Gold standard. 30 CKD cases were recruited from nephrology OPD. 30 age matched control subjects were recruited for the study. Urea, creatinine and albumin were estimated for all sixty subjects. GFR was estimated using Gates method, equation. A comparative study was done using the Bland and Altman analysis method. For control subjects Cockcroft Gault estimation was more superior to MDRD, as MDRD was underestimating GFR as evidenced by the r value. For CKD patients, Cockcroft Gault estimation was better as evidenced by r value and SD. In the Indian ethnic population Cockcroft and Gault algorithm gives better accuracy and precision as compared to MDRD for both normal as well as CKD patients.

S130

Assessment of Serum Osteocalcin in Postmenopausal Women of Lucknow

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Osteoporosis (OP) is a chronic, progressive disease characterized by low bone mass and microarchitectural deterioration of bone, resulting in increased risk of fracture. In India, it affects approximately one in three women. Because of related morbidity, disability, diminished quality of life, and mortality, OP and fractures associated with it are major public health concern. A lack of estrogen in postmenopausal women prevents the absorption and utilization of calcium and is the single most important factor in the development of OP in older women. Osteocalcin is a promising marker of bone turnover useful in the diagnosis and follow-up of high turnover osteoporosis. Objective was assessment of serum osteocalcin in postmenopausal women of Lucknow. The patients visiting the department of Orthopaedic Surgery Era's Lucknow Medical College, Lucknow, were recruited for the study after Institutional ethics committee approval. Total 41 women with OP (aged 40-60 years) and 41 controls (age and sex matched) were taken. All the subjects were subjected to DEXA for bone mineral density, while Osteocalcin was estimated using ELISA kit. Mean bone mineral density *T* score was significantly decreased (P< 0.001) in postmenopausal OP patients as compared to controls. Serum osteocalcin levels were significantly increased (P< 0.001) as compared to control group. Serum osteocalcin is a promising marker of bone turnover in post-menopausal women with OP, as it was found elevated in OP. Thus, Osteocalcin provides a dynamic measure of bone remodelling and it can be potentially useful in diagnosis and monitoring of response to therapy in patients of osteoporosis.

S131

Arterial Blood Gas (ABG) Analysis in Chronic Obstructive Pulmonary Disease

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Arterial Blood Gas (ABG) analysis PaO2, PaCo2, pH and Bicarbonate is important in diagnosis, prognosis and medical management of Chronic Obstructive Pulmonary Disease (COPD). ABG analysis also important for Anaestehtist to monitor the mechanical ventilator setting in I.C.U. Interpretation of PaO2, PaCo2, pH and HCO3- are considered respiratory and metabolic parameter and useful in Acid-Base disorders. Choice of respiratory and metabolic parameter (ABG) specially in COPD are important due to respiratory Acidosis and respiratory Alkalosis. Arterial Blood Gas (ABG) analysis in 30 patients (Male and Female) with lung disease (COPD) admitted to ICU were studied. ABG Analysis was done by ABG Analyzer (Cobas b-121, Roche Omni C, Japan). PaO2, PaCo2, pH and HCO3- evaluated in COPD patients. Blood gas pressure and acid base state in the arterial samples were compared with healthy control group of 30 cases with paired t test. In this studied the levels of PaO2, PaCo2, pH and HCO3- in COPD patients were estimated and values compared with healthy control group. The mean value with S.D. of PaO2 (mmHg) was found to be 50 ± 1.80 with (P< 0.001), PaCo2 (mmHg) was found to be 60 ± 1.50 with (P< 0.001), pH was found to be 7.30 ± 0.05 with (P< 0.005) and HCO3 (mmol/L) was found to be 30.5 ± 3.8 with (P< 0.001). Interpretation of ABG is relevent and sensitive parameter for clinician and Anaesthetist. In COPD, ABG analysis and acid base state are significant in present study.

A 001

Efficacy of *Emblica officinalis* (Amla) in Arsenic induced Oxidative Stress and Pro-inflammation in Hepatocytes of Mice

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Arsenic is widely distributed in the environment and has been found to be associated with the various health related problems. The fruitextract of *Emblica officinalis* (amla) has been shown to have anti-oxidants and anti-inflammatory properties. Inview of increasing health risk of arsenic, the present study has been carried out to investigate the protective effect of amla against arsenic induced oxidative stress and pro-inflammation of mice. The present study was carried out to investigate anti-oxidants, anti-inflammatory and hepato-protective effects of Amla against arsenic induced hepatotoxicity in mice. The Balb/c mice were treated with arsenic (3mg/kg body weight/day,P.O.) and simultaneous given fruit extract of amla (500mg/kg body weight/day, P.O.) for 30 days. Estimation of arsenic levels in hepatocytes and serum biomarker of hepatic dysfunction (SGOT & SGPT) and oxidative stress as well aspro-inflammatory (IL-β, IL-6, TNF-α) markers were done and compared to control. Arsenic exposure in mice caused a significant change in serum biomarkers (SGOT & SGPT) and enhanced IL-β, IL-6, TNF-α level and simultaneously alternation of oxidative stress in hepatocytes as compared to control groups ($P<0.05$). Interestingly, simultaneous exposure of arsenic and fruit extract of amla caused significantly decrease in the serum SGOT, SGPT and pro-inflammatory markers in serum as compared to arsenic alone ($P<0.05$). The simultaneously exposed group also reduced level of oxidative stress as well as arsenic level in hepatocytes as compared to the arsenic alone group. No significant changes in these parameters following exposure to amla alone group as compared to control group. The results of the present study suggest the antioxidant and anti-inflammatory property of amla that could be utilized for its protective efficacy in arsenic induced hepatotoxicity.

A002

Anemia and Lower Magnesium Levels at The Initiation of Tuberculosis Therapy is Associated with Delayed Sputum Conversion Among Pulmonary Tuberculosis Patients

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Pulmonary tuberculosis and anemia are both prevalent in India. Magnesium levels also influences TB. There is limited and inconsistent literature on the association between anemia, serum magnesium levels and sputum conversion following tuberculosis treatment. Newly diagnosed 100 sputum smear positive pulmonary tuberculosis and 100 sputum smear negative patients initiating on DOTS therapy were recruited. Patients were followed up prospectively until completion of first two months of intensive phase. Patients were evaluated before initiation of TB treatment by performing the following complete blood counts including peripheral blood smear, serum biochemistry, serum iron, magnesium, ferritin and sputum microscopy. Sputum smears were re-examined at two months of anti-tuberculosis therapy for presence of acid fast bacilli. Anemia was defined as hemoglobin <13 g/dl (males) or, 12 g/dl (females). Serum iron and serum ferritin was significantly lower in sputum positive PTB as compared to sputum negative PTB and controls. Anemia was present in 162 (81%) patients of the study PTB cases. 60% of anemia in sputum positive cases was iron deficiency anemia. Serum magnesium level was significantly lower in sputum positive PTB as compared to sputum negative PTB and controls though not in hypomagnesemic range. Delayed sputum smear conversion occurred in 12 (12%) sputum positive PTB patients. Of these 8 had severe iron deficiency anemia, 4 with moderate anemia. All 12 delayed sputum smear conversion had serum magnesium levels < 1.7 mg/dL (below normal reference range) (mean 1.42 $\pm$0.22 mg/dL). Baseline anemia and lower serum magnesium levels are associated with increased risk for persistent positive sputum smears at two months of tuberculosis treatment. Future studies should evaluate the mechanisms for TB-associated anemia as well as the role of intervention for anemia among TB patients.

A003

Vitamin B12 Deficiency is Associated with Dyslipidemia, Insulin Resistance and Inflammatory Markers in Indians with Coronary Artery Disease

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Folate and vitamin B12 are essential components in the metabolism of homocysteine (Hcy). Hyperhomocysteinemia has been implicated in endothelial dysfunction and cardiovascular disease. However, the association of Hcy, vitamin B12 and folic acid with dyslipidemia, insulin resistance and inflammatory markers in patients with coronary artery disease has not been studied in Indian patients. This study was conducted with aim to evaluate the relationship of vitamin B12, folic acid and Hcy levels with cardiovascular risk factors especially dyslipidemia, insulin resistance and inflammatory markers in subjects with known CAD. Three hundred patients (M:216; F:84, age:25-92) who had coronary disease on angiography were included in this study consecutively. All patients were evaluated for anthropometry and cardiovascular risk factors, and blood samples were collected for biochemical and inflammatory markers. Percentage of vitamin B12 and folate deficiency was 86.7% and 2.7% respectively. Hyperhomocysteinemia was present in 95.3% patients. Vitamin B12 levels were significantly lower and Hcy levels were significantly higher in subjects with dyslipidemia, DM and/or HTN. Serum vitamin B12 was inversely associated with triglyceride, VLDL and positively with HDL. Hcy was positively associated with triglyceride, VLDL and negatively with HDL. Vitamin B12 was inversely correlated with inflammatory markers (hsCRP and IL-6) and directly related to insulin resistance whereas Hcy showed opposite pattern. Serum vitamin B12 deficiency and hyperhomocysteinemia are related with dyslipidemia, insulin resistance and inflammatory markers in Indian patients with coronary artery disease.

A004

Antagonistic Effect on Nutlin-3a in Male Fertility via UQCRC2

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Ubiquinol-cytochrome-c reductase core protein 2 (UQCRC2) is a component of ubiquinol-cytochrome c reductase complex that is known to correlate with male fertility via spermatogenesis. Simultaneously, nutlin-3a is a small molecule antagonist of mouse double minute 2 repressor (MDM2), activate p53 and induce apoptosis responsible for spermatogenesis. To date, however there are no known effects of nutlin-3a on reproduction. Therefore, present study was designed to investigate the effect of nutlin-3a on male fertility via UQCRC2. In this in vitro trial with mice spermatozoa, we utilized CASA, CTC staining, ATP assay, western blotting, and IVF to measure the main study outcome. The short-term exposure of spermatozoa in nutlin-3a decreases sperm motion kinematics, intracellular ATP production, capacitation, the acrosome reaction, UQCRC2, and tyrosine phosphorylation (TYP) of sperm proteins in a dose-dependent manner. Notably, the decreased UQCRC2 and TYP were associated with reduced sperm kinematics, ATP production, and capacitation, which ultimately led to adverse effects on male fertility such as poor fertilization rates and embryo development. Thus, nutlin-3a may be considered as a potential male contraceptive agent due to its ability to decrease fertility secondary to changes in overall sperm physiology and embryonic development. However, the results of this preliminary study have to be confirmed by additional independent trial

A005

Serum Anti-Müllerian Hormone Levels and its Correlation with BMI in Breast Carcinoma Patients

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AMH is a protein hormone structurally related to inhibin and activin and is a member of the transforming growth factor-β (TGF-β) family. AMH is expressed by granulosa cells of the ovary during the reproductive years and controls the formation of primary follicles by inhibiting excessive follicular recruitment by Follicular Stimulating Hormone (FSH). Aim was to estimate serum AMH levels in Breast carcinoma patients and to study the correlation between BMI and serum AMH levels.The present study was

conducted in the Department of Biochemistry in collaboration with Department of Radiotherapy, Pt. B.D. Sharma, University of Health Sciences, Rohtak between June 2013 and June 2014. Thirty female patients of confirmed diagnosis of breast carcinoma shall be enrolled in the study group (Group I). 30 healthy age matched female volunteers were enrolled as controls(Group II).Females on oral contraceptive pills / hormone therapy or drugs affecting hormone levels were excluded from the study. The serum AMH levels ingroup I was 1.67 ± 0.44 ng/ml which was significantly lower as compared to group II was 1.9 ± 0.37 ng/ml (P< 0.01). Although there was a negative correlation between BMI and serum AMH levels in breast carcinoma patients (group I) (r= -0.206) but it was not statistically significant (P>0.05). Serum AMH levels in breast carcinoma patients were decreased as compared to healthy controls. Also there was an inverse relationship in Serum AMH and BMI in breast carcinoma patients indicating decrease AMH levels with increased BMI.

A006

Tobacco Exposure by Various modes may Modulate pro-(IL-12) and Anti-(IL-10) Inflammatory Levels and Influence the Median Survival of Prostate Carcinoma Patients: An Explorative Study in North Indian Population

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Inflammation is an important hallmark of all cancers and net inflammatory response is determined by a delicate balance between pro- and anti-inflammatory cytokines, which may be affected by various environment factors including tobacco exposure. The present study was designed to explore the effect of various modes of tobacco exposure on Interleukin-12 (IL-12) and Interleukin-10 (IL-10) inflammatory cytokine levels and survival in prostate carcinoma (PCa) patients. 285 cancer patients and equal controls with 94 BPH (Benign Prostatic Hyperplasia) were recruited; baseline levels of serum IL-12 and IL-10 were measured by ELISA kits and analyzed in various tobacco exposed groups by appropriate statistical tool. Five year survivals of patients were analyzed by Log-rank (Mantel-Cox) test (graph pad version 5).The expression of serum pro-inflammatory (IL-12) among tobacco smokers groups showed unique trend i.e. the levels were highest in bidi's smokers, followed by chillum, cigarette, hookah (bidi's smokers > chillum>cigarette smokers >Hookah). Further in tobacco chewers the levels were highest in khaini chewers, followed by gutkha, betel quid chewers (khaini > gutkha >betel quid chewers. Moreover pro-(IL-12) and anti-inflammatory (IL-10) weresignificantly higher (P<0.001) in smoker, chewer and alcohol users (combined users), with significant low median survivals (27.1 months, standard error=2.86 and 95 % CI: 21.4-32.62) than non-users. Stage (III and IV) of tobacco addicted patients have also shown significantly increased levels of IL-12 and IL-10. IL-12 and IL-10 seems to be influenced by various modes of tobacco exposure and inflammation also affects median survival of cancer patients.

A007

C-peptide and apo-proteins - Novel markers of cardiovascular disease risk in Hypothyroidism

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An ever increasing incidence of cardiovascular disease (CVD) in hypothyroidism seeks novel biochemical markers that could help in the early detection of the of this disease complex.The present study aimed at evaluation of CVD risk in newly diagnosed hypothyroid subjectsusing novel markers C-peptide and apo-proteins.We included 100 healthy controls and 150 hypothyroid subjects andwere evaluated anthropometrically, clinically (Blood pressure) and biochemically (Fasting Blood Sugar (FBS), Lipid profile, C-peptide, apo – proteins). Statistical analysis was done using the student's't' test and Pearson's coefficient of correlation. The newly diagnosed hypothyroid subjects presented with significant obesity in both males and females (P<0.001), hypertension (Systolic blood pressure (SBP) P<0.001) (Diastolic blood pressure (DBP) P<0.001), fasting hyperglycaemia (142.49 ±65.07 mg/dl v/s 83.53 ±11.23 mg/dl P<0.001), dyslipidaemia, raised Apo – B (175.58 ±34.56 mg/dl v/s 82.9 ± 10.94 mg/dl, P<0.001) and C – peptide (3.45±1.93 ng/ml v/s 1.46±1.08 ng/ml, P<0.001) levels and reduced Apo $-A_1$ (139.76±17.40 mg/dl v/s 140.75 ± 10.20 mg/dl, P<0.001). Statistically significant association of C – peptide was observed with SBP (P=0.016), DBP (P=0.011), FBS (P<0.0001), total cholesterol (P<0.0001), TG (P<0.0001), low density lipoprotein (P<0.0001), Apo – B (P<0.0001) and CVD risk ratiosTCH/HDL (P=0.001) and apo – B/ Apo – A1 (P=0.006). Serum C – peptide and apo-proteins in hypothyroid subjects at diagnosis can serve as a novel marker for an early evaluation of

CVD risk of these patients.

A008

Is Dylipidemia a Risk Factor for Microalbuminuria in Normotensive Type 2 Diabetes Mellitus?

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Diabetes is pandemic with an estimated increase in number globally to an alarming level of 79.4 million by 2030 According to a population based survey the prevalence of overt nephropathy was found to be 2.2% and that of microalbuminuria was 26.9% in India. Aim was to identify contributing risk factors in development of microalbuminuria in type 2 diabetes mellitus [T2DM]. The study comprised of total 97 normotensive T 2 DM patients with 50 age and gender matched heathy controls.The normotensive diabetes patients were further grouped into group 1 and group 2 based on presence of microalbuminuria. Venous blood sample was taken to estimate blood glucose, lipid profile, glycated haemoglobin, and malondialdehyde [MDA] with literature approved standard techniques.The normotensive T2DM patients had significantly deranged lipid profile in comparison to healthy adults. The triglyceride levels were significantly different [P<0.05] between normotensive T2DM without microalbuminuria [Group 2] and normotensive T2 DM with microalbumiuria [group 3]. Rest of the parameters like total cholesterol, LDL were higher and HDL lower in group 3 in comparison to group 2 but the difference was individually not significant [P>0.05]. Similarly fasting blood glucose and HbA1c% were individually not significantly different between the two subsets of normotensive T2DM patients. MDA was significantly different between the three groups [P<0.01]. Albumin creatinine ratio [ACR] was correlated with all the parameters of lipid profile, MDA, FBS and HbA1c% in normotensive T2DM with microalbuminuria and significant correlation was seen between ACR & MDA [r=0.795, P value<0.001], ACR & HbA1c% [r=0.799, P value<0.001], ACR & FBS [r=0.749, P value<0.001], and ACR & cholesterol [r=0.716, P value<0.001] ACR &LDL [r=0.676, P value<0.001] LDL&MDA [r=0.595,P<0.001]. Dyslipidemia in addition to hyperglycemia contributes to oxidative stress contributing to microalbuminuria in normotensive T2DM patients.

P 001

A Study on Aldehyde Dehydrogenase (ALDH2) Polymorphism (rs671) in Alcohol Dependent Subjects of Sikkim

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Alcohol dependence is a chronic relapsing health disorder and is a major cause for morbidity and mortality worldwide. Several genetic polymorphisms have been linked with this disease, among which ALDH2 polymorphism (rs671) seems to be most strongly associated. A variant of aldehyde dehydrogenase encoded by ALDH2*2 allele is said to be protective against development of alcohol dependence. But with recent surge in the socio-cultural promotion for alcohol consumption and some existing contradictory literature, the protective effect of the same allele needs to be re-evaluated. The objective was to characterize the ALDH2 (rs671) polymorphism among alcohol dependent subjects attending Central Referral Hospital, Sikkim.This was a hospital based candidate gene study. 50 alcohol dependent subjects of either sex in the age group of 18-60 years were selected as cases. History, laboratory investigations and demographic profile were noted. 2 ml of venous blood was collected in EDTA (anticoagulant) tube under aseptic measures with due consent. Genomic DNA was extracted, quantified and ALDH2 (rs671) genotyping was done by a polymerase chain reaction (PCR) based restriction fragment length polymorphism (RFLP) technique. All of the participants were found to be homozygous for ALDH2*1 allele. The minor allele (ALDH2*2) was not found in the study population. The absence of the minor allele from the study population supports the existing hypothesis of its protective role against development of alcohol dependence.

P 002

Adenosine deaminase activity and CRP in serum of patients of alcoholic liver disease- a useful tool in monitoring clinical status

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Alcoholic liver disease is due to the chronic inflammatory and toxic effects of ethanol on the liver. Acute alcoholic hepatitis occurs in habitual drinkers, often a period of increased alcohol intake. Progression of liver fibrosis to cirrhosis is dangerous as well as potentially life threatening condition that occurs when inflammation and scarring damage. Several biochemical parameters are altered in this condition. A combination of tests are useful for diagnosis of cirrhosis. The evaluation of adenosine deaminase (ADA) and C-Reactive protein (CRP) in sera of patients of alcoholic liver disease should be considered a useful tool in the monitoring of their clinical status. In this study, we aimed to determine the relationship between serum ADA level and CRP levels in Alcoholic liver disease. This group will include 50 clinically diagnosed cases of alcoholic Liver diseases supported with serological tests, ultrasonogram in the age group of 25-60 years with age and sex matched healthy control. Serum adenosine deaminase was done by the method of Giusti and Galanti . The estimation of C-reactive protein was done by Immunoturbidimetric method. Mean of ADA cases was 66.5±4.3U/L and control was 6.14±0.36U/L and $P<0.001$ and mean of CRP cases was 20.4±0.74 mg/L and control was 6.7±0.07 mg/L and $P<0.001$. ADA and CRP levels were significantly increased in alcoholic liver disease patients. Evaluation of Adenosine deaminase and CRP are potentially useful prognostic markers for the assessment of alcoholic liver disease.

P 003

Detection of New Human Metabolic Urinary Markers in Chronic Alcoholism and their reversal by Aqueous Extract of *Tinospora Cordifolia* stem

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Chronic alcoholism (CA) leads to a wide variety of alcohol use disorders for which no effective treatment is available.The aim of the study was to study urine metabolic signature of CA before and after treatment with an Ayurvedic drug '*Tinospora cordifolia*' aqueous extract (TCE). Urinary metabolites of chronic alcoholics and apparently healthy subjects were profiled using HPLC-Q-TOF-MS. Five different types of discrimination models were constructed and trained and used for prediction of unknown samples. Common metabolite on basis of their ranking and used in models were characterized and used as bio-marker of CA. Discrimination models from the initial data sets were able to correctly assign the unknown samples to the CA, treated or healthy groups in validation sets with $r^2 > 0.9$. Metabolic signature in CA patients include changed tryptophan, fatty acids and pyrimidines metabolism. Several novel biomarkers of alcoholism were observed in urine for the first time which includes: 5-hydroxyindole, phenylacetic acid, picolinic acid,quinaldic acid, histidine, cystathionine, riboflavin, tetrahydrobiopterin andchenodeoxyglycocholic acid in addition to previously reported biomarkers. Treatment of CA with TCE reverted the levels of most of biomarkers except tetrahydrobiopterin levels. These results suggested that the measurement of these urine

metabolites could be used as a non-invasive diagnostic method for detection of CA. As TCE treatment significantly reversed the affected pathways without any side effect, it may be a useful as stand alone or adjunct in reducing alcohol induced disorders.

P004

Recurrent Pancreatitis and Its Sequale in Chronic Alcoholic

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Acute pancreatitis remains a common disease occurring in alcoholics. Chronic alcoholism leads to recurrent pancreatitis, which in turn leads to pancreatic pseudocyst in 5-16% of the cases. A 58 year old male, chronic alcoholic, presented with complaints of hiccups, severe abdominal pain and fever of 1 day duration. On examination, diffuse abdominal tenderness and epigastric pain radiating to back with mild hepatomegaly was noticed. Guarding was noticed in the epigastric region. Patient also showed psychotic behaviour and had a history of similar episodes in the past. Lab values revealed markedly elevated serum amylase (884 U/L) and lipase levels (2324 U/L). Liver function tests and CA 19-9 was also elevated. Based on initial work-up, a diagnosis of Acute Pancreatitis was made.USG abdomen revealed bulky head and body of pancreas showing multiple pseudocysts of varying sizes (largest 10x7cm) and dilated pancreatic duct, suggesting features of acute pancreatitis and its sequale. CT scan abdomen revealed acute pancreatitis with multiple pseudocysts (largest being 10x5.5 cm). Thus, a diagnosis of Pancreatic pseudocyst as a sequale of acute pancreatitis was made. The pseudocysts were drained and conservative management was initiated simultaneously. Drainage Fluid analysis from pseudocyst revealed albumin of 0.6 g/dl and fluid amylase of 102245 U/L. Recurrent acute pancreatitis in a chronic alcoholics leads to complications like pancreatic pseudocyst. Interesting finding in our case was highly elevated serum amylase and lipase levels helping in the diagnosis of acute pancreatitis and enormous amount of amylase (*102245 U/l) in the drainage fluid was an unusual finding. If the pseudocyst is >5 cm size and > 8 weeks duration as in this case, intervention like surgical drainage should be considered along with conservative treatment.

P005

Tinospora cordifolia Modulated Peroxisome Proliferator-activated Receptors in Alcoholics

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Peroxisome proliferator-activated receptors (PPARs) are associated with fatty acid catabolism and are prime targets for treating hyperlipidemia. The present study was conducted to explore relationship between PPAR and in development of hyperlipidemia in alcoholics and effect of treatment of fresh juice of *Tinospora cordifolia* (TCJ) in hyperlipidemic (HPL) patients. 42 volunteers (36-40 years) were prescribed fresh TCJ (100 ml) in the early morning with empty stomach for 14 days in a randomized two-way crossover intervention study. Urinary excretion levels of pantothenic acid, amino acids and acylcarnitine were studied by HPLC-QTOFMS at 0 and 14 day of treatment. At the same time, traditional biomarkers of hyperlipidemia and γ-GT, *SGOT*, SGPT and MCV along with other biochemical parameters were also analyzed. PLSDA of RRLC-QTOF-MS data revealed the decreased urinary secretions of PPARα and PPARα biomarkers in HPL patients after TCJ treatment. Interestingly, the levels of newly reported acylcartinine; L-hexanoylcarnitine and 4-hydroxyisovaleric acid were also observed low by two fold after TCJ treatment as compared to non-treated HPL samples. Besides this, the biomarkers of HPL; ursodeoxycholic acid, chenodeoxycholic acid and hippuric acid were also observed depleted after TCJ treatment. Through biochemical study, the other conventional markers of HPL; triglyceride, cholesterol and LDL levels were also found reduced in TCJ treated HPL patient samples.The present data clearly depicts the regulatory effects of TCJ on the lipid metabolism and also emphasize that TCJ contains PPAR agonists which may further responsible for decreasing the urinary levels of PPAR biomarkers.

P006

Inflammatory Markers (Intergenotypic Variation) in Coronary Artery Disease

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Coronary artery disease (CAD) is rapidly invading developing countries. It is the end result of atherosclerosis in which inflammatory process has a central role. Patho-physiology of CAD is highly influenced by genetic factors.The aim of this study was to look for a relationship between the C150T polymorphisms of

the iNOS and inflammatory markers in stable CAD patients. A total of 300 subjects (150 patients with stable CAD and 150 controls were included in this case-control study. Their blood samples were analyzed for IL-2, IL-2R, IL-6 and TNF-α and iNOS gene polymorphism. Levels of inflammatory markers (IL-2, IL-2R, IL-6 and TNF-α) in study group were significantly higher compared with controls (P<0.001). In study group, these levels were significantly higher in CT genotype compared with CC genotype (P<0.0001). Genotype/allele distribution was significantly different among groups [CC, 115 (77%) vs 124 (83%); CT, 35 (23%) vs 26 (17%); respectively in study and control groups; (P<0.0001)]. Inflammation plays a major role in CAD. The presence of T allele might be associated with its increased risk and poor prognosis.

P007

Effect of *Tinospora cordifolia* Juice Treament on Fatty Acide Amides in Alcohol Abuse

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Alcoholism modulates the activity of fatty acid amide hydrolases that degrades the fatty acid amides.The aim was to observe differential accumulation of fatty acid amides (FAA) in alcoholic person and effect of *Tinospora cordifolia* (Willd) Mier. (Menispermaceae) juice on FAA.12 asymptomatic chronic alcoholics (39 ± 2.0 years old) without chronic liver disease and 14 non-alcoholic healthy volunteers were selected for all studies. For various biochemical profiles such as transaminsase activities, gamma-glutamyltransferase (γGT), red cell mean corpuscular volume (MCV) and lipid profiles, blood samples were collected. This study was performed by using Q-TOF-MS, an accurate Mass Spectrophotometer. Data analysis identified six FAA; oleamide, myristamide, palmitamide, linoleamide, stearamide, and erucamide. Oleamide and palmitamide were reported high in alcoholic samples but after treatment with *T. cordifolia* level of these compounds got reverse. After TCJ Treatment the level of all six identified FAA were found to be down-regulated. Hence, fatty acid amides can be used as a biomarker of several diseases in subjects. As TCJ was found to have reversion effect in alcoholism, it may be used as herbal drug for alcoholic abuse as well as curing other diseases.

P008

Cognitive Performance & MDA Levels among the Rats Exposed to Alcohol During Fetal Life

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Neurons in the brain are highly sensitive to oxidative stress. Metabolic products of alcohol consumed by mother can induce oxidative stress in fetal brain causing cognitive impairment in offsprings.The aim was to compare effects of prenatal ethanol exposure on locomotor activities, memory retention and MDA level in the brain homogenate of male and female offsprings. Female rats were divided into control and alcoholic group. Rats in alcoholic group were orally fed with 30% alcohol (5g/kg body weight, single dose /day), which is started 14 days before mating, continued throughout their gestation and weaning period. Control group were administrated with equivalent volume of water. Offspring from each group were separated into male and female group. Behavioral studies started on 75th day of post natal life, animals were sacrificed on 90^{th} day of post natal life and brain tissue homogenate was prepared for estimation of MDA level. Statistical analysis was done by independence T test. Offspring from alcoholic mothers showed decreased memory retention (P<0.001) as compared to the offspring from normal mothers and it was significantly more in female offspring (P<0.05). In Open field test offspring from alcoholic mother showed increased locomotor activities as compared to normal offspring (P<0.05) and it was significantly more in females (P<0.001). MDA level in the brain tissue is more in offspring from alcoholic mother (P<0.01) as compared to normal and it is more in female offspring(P<0.000). The maternal consumption of alcohol during pregnancy has greater adverse effect in young adult female offsprings, revealing their increased susceptibility to oxidative stress.

P009

^{18}O-isotope of Breath CO_2: A Potential Non-invasive Marker of *Helicobacter Pylori* Infection

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Helicobacter pylori (*H.pylori*), the most frequent bacterial infectious disease in human stomach, is diagnosed based on endoscopy and the biopsy-based rapid urease test (RUT), bacterial

culture and histopathology. At present, ^{13}C-urea breath test (^{13}C-UBT) is performed for non-invasive detection of *H. pylori* infection. As the oxygen-16 isotope in $^{12}C^{16}O_2$ and the oxygen-18 isotope of body water ($H_2{}^{18}O$) are rapidly exchanged to produce $^{12}C^{16}O^{18}O$ (^{18}O of CO_2), catalyzed by carbonic anhydrase (CA) metalloenzyme (*H. pylori* contains α-CA and β-CA), the aim of the present study was to find out the potential link between ^{18}O-isotope of breath CO_2 and *H. pylori* infection. Breath samples were collected at baseline and 15 min intervals for upto 60 min following the injection of non-radioactive 75 mg ^{13}C-labelled urea with 4.0 g citric acid dissolved in 200 ml of water. The breath samples were analyzed to determine both the $^{18}O/^{16}O$ and $^{13}C/^{12}C$ isotope ratios of CO_2 by a high-resolution carbon dioxide isotope analyzer based on off-axis integrated cavity output spectroscopy (ICOS). We found both $^{18}O/^{16}O$ and $^{13}C/^{12}C$ of exhaled CO_2 (expressed as $\delta_{DOB}{}^{18}O‰$ and $\delta_{DOB}{}^{13}C‰$ respectively) reached peak values from their basal values at around 30 min during ^{13}C-UBT and then slowly decreased, whereas no significant changes were observed for individuals with *H. pylori* negative. Next, we performed UBT with unlabelled urea and observed the similar excretion kinetics patterns of $^{18}O/^{16}O$ of breath CO_2 from its basal value in *H. pylori* positive individuals. Further, using receiver operating characteristic curve analysis, the optimal diagnostic cut-off point of the $\delta_{DOB}{}^{18}O‰$ value in exhaled breath samples was determined to be 1.92‰ for precisely distinguishing *H. pylori* positive and negative individuals.

P010

DNA Methylation in Head and Neck Cancers

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Hypermethylation of promoter regions of tumour suppressor genes is known to cause down regulation of tumour suppressor genes. We evaluated DNA methylation of two tumour suppressor genes p15 & E cadherin on histopathologically proven cases of head and neck cancers.The objective was to study methylation in the promoter region of p15 and E Cadherin genes from tissue samples of head & neck cancer and compare it with peripheral blood. Forty three cases of head & neck cancer were taken as study subjects. DNA was extracted from HPE proven head & neck cancers. This DNA was bisulphite modified. Methylation Specific PCR was done on the modified DNA to detect methylation pattern. DNA was extracted from peripheral blood of the same patients and the same procedure was followed on the modified DNA to compare the methylation patterns in head & neck cancer tissue as well as in the blood. DNA methylation was detected in 49% tissue samples and 40% peripheral blood samples in p15 gene & 63% tissue samples and 44% peripheral blood samples in E cadherin gene. Kappa coefficient is 0.346 for p15 gene and 0.277 for E cadherin.DNA methylation of promoter region of E cadherin & a p15 tumour suppressor gene which causes them to become downregulated may be one of the mechanisms of development of head & neck cancer. This is a reversible mechanism. Demethylating agents like Azacytidine and Decitabine can be exhibited to patients of Head & neck carcinoma who show evidence of DNA methylation.

P011

Analysis of Clinical and Serum Proteome Profiles of Vivax and Falciparum Malaria in a Tertiary Care Centre in Mumbai

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India has the largest population in the world at risk of malaria. Investigation of parasite induced alterations in hematological and biochemical parameters and host proteome can enhance our understanding of disease pathogenesis and host-parasite interactions in malaria.The present study has been conducted to perform an in-depth comparative analysis of clinical and serum proteome profiles of vivax and falciparum malaria in a tertiary care centre in Mumbai for studying malaria pathogenesis. In this study serum samples from confirmed cases of non-severe vivax (n=15) and falciparum malaria (n=15) along with community controls (n=30) were analyzed. Hematological and biochemical parameters were measured in malaria patients and healthy controls. Two-dimensional gel electrophoresis (2-DE) was employed for differential proteomic analysis and identification of proteins that differentially expressed has been performed using high-sensitivity MALDI-TOF/TOF mass spectrometry. For revealing the presence of phosphoproteins in the malaria and control sera, the 2D gels were stained using a Pro-Q® Diamond Phosphoprotein Gel Stain. Among hematological and biochemical parameters platelets, SGOT and ALP were found to be significantly altered in malaria cases ($P = 0.01$) in comparison to healthy controls. The expression levels of quite a few serum proteins, including serum amyloid A, haptoglobin, and hemopexin were found to be altered significantly ($P< 0.05$; Fold-change > 1.5) in the malaria patients. Among the differentially expressed proteins, hemopexin was found to be phosphorylated in malaria. Hemopexin forms complex with hemeproteins and induces intracellular antioxidant activities. As during the malaria infection, hemoglobin degradation produces globin free heme and certain hemoproteins, which can act as active triggers of low-density lipoprotein (LDL) peroxidation.

P012

Efficiency Gains Due to Total Laboratory Automation in the Clinical Chemistry Laboratory

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In 2011, the clinical laboratory at our institute installed a system called Total laboratory Automation (TLA). The same year it got certified by National Accreditation Board for Testing and Calibration Laboratories (NABL). The aim was to show the benefits of TLA through improved turnaround time (TAT), predictable process times, improved result accuracy, coping with increased workload across all shifts without an increase in staff. Data was extracted from Laboratory Information System (LIS) for TAT for electrolytes, result finalization time outliers (March 2014-September 2014) and for workload increase (2011-2013). Target TATs for samples from the Emergency department and ICU were 100% reported in 2 hours. These were stat samples. Only 6 out of 8376 samples were delayed. All other samples were considered routine, and target TAT for them was 100%, results reported in 6 hours. Only 12 out of 141315 were delayed. For Internal Quality Control (IQC), 2 levels of Bio-Rad Lymphochek Assay is run 2 times daily. Material for External Quality Assurance Services (EQAS) is obtained from Bio-Rad and data is submitted once per month.The workload has increased by 48,100 tests in 2 years without an increase in manpower. Other advantages of TLA: a) Increase in the spectrum of tests several fold in the limited time and space b) lower laboratory errors during pre-analytical, analytical and post-analytical processes c) Speedy delivery of reports through HIS d)decreased waste generation e) Increased staff safety due to minimal human contact with potentially infected materials f) The scientific staff can spend more of their time reviewing and validating abnormal patient results and quality control.

P013

Correlation of Serum Nitric Oxide level with First Trimester Screening Dual Markers

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Free β hCG is a hormone produced by the syncytiotrophoblast of the placenta. Generally maternal free βhCG level rises till 10^{th}week and start to decline from 16^{th} week onward. It is a marker of first trimester screening. PAPP A is a glycoprotein derived from syncytiotrophoblast. PAPP A is a widely used marker of first trimester screening. NO is a major paracrine mediator and important regulatory agent in various female reproductive process such as ovulation, implantation, pregnancy maintenance, labour and delivery. The objective of the study was to correlate serum nitric oxide level with first trimester screening dual markers. The study group comprises of 50 pregnant women within the age of 18-35 years with singleton pregnancy who came for routine first trimester screening. Serum levels of free β hCG and PAPP A were analysed by ECLIA. NO was measured by kinetic cadmium reduction method.We found significant($P>0.01$) increase in the level of NO in pregnant women and there was a positive correlation between NO and free β hCG. In this study we found there was a positive correlation between serum NO level with free β hCG. An in-depth study with higher number of subjects is needed to conclude the result of correlation between serum NO and first trimester screening dual markers.

P014

Antitryptic Acitivity in Chronic Hypertension and Pre eclampsia

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Antitryptic activity(AA) of blood plasma is mainly contributed by α1- antitrypsin, α1-antichymotrypsin and α2-macroglobulin. Of late, the role of anti-proteases in regulating multiple events of gestation from implantation to term is becoming evident. It is being explored in an effort to use enzyme inhibitors as an avenue of therapy in gestational complications. The aim was to investigate and compare AA in blood plasma of normal nonpregnant (NNP), normal pregnant (NP), hypertensive pregnant (HP) and preeclamptic (PP) pregnancies in the last trimester. Estimation of AA in plasma, NNP (n= 25) and NP (n=20), HP with blood pressure above 160/100,(n=20), and PP(n=20) with proteinuria ('+' with urine dipstick) was estimated by the Kunitz caseinolytic method. Mean and standard deviation and 'P' values were calculated using GPIS software. A in NNP was 108±30 units/ml and increased in NP to 220 ±35 units/ml and HP to 264±32 units/ml in the third trimester. In PP it was lower than in NP (180±42 units/ml). The rise in AA in NP, HP and PP was highly significant ($P<0.001$) when compared to NNP and NP. The difference in AA between HP and PP was also significant ($P<0.01$).The fact that AA changes in NP, HP and PP indicates that it has a major role to play in regulating the proteolytic events during these conditions. A detailed investigation with subdivision of target group into mild, moderate and severe, based on blood pressure and proteinuria is warranted.

P 015

Study of Anthropometric, Hormonal and Biochemical Profile Among Obese and Non – Obese Women with Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder characterized by chronic anovulation, hyperandrogenism, multiple small subcapsular cystic follicles in the ovary. PCOS is associated with metabolic abnormalities - insulin resistance, metabolic syndrome, dyslipidemia, besides increased risk for cardiovascular diseases. Earlier, it was believed that only obese women are susceptible to PCOS. Recent evidence shows that many non –obese women are also developing PCOS.To investigate the anthropometric, biochemical, hormonal profile among obese and non – obese women with PCOS.150 women with PCOS, divided into two sub – groups depending on body mass index (BMI) (<> 30 kg/m^2), along with 75 healthy age – matched control women were studied. Serum lipid profile, fasting blood glucose, fasting insulin levels, thyroid profile, luteinizing hormone, follicle stimulating hormone, and prolactin were measured. Statistical analysis was done using SPSS software 20.0. There were about 30% of PCOS women with normal BMI. High fasting blood glucose and insulin levels, high cholesterol, triglyceride, LDL, VLDL and lower HDL levels were found in both obese and non obese PCOS compared to control women. Similarly lower FSH level, higher LH levels and LH/FSH ratio, mild to severe hyperprolactinaemia was found in the both the groups when compared to controls. There was significant difference in lipid profile levels and gonadotropin hormone levels. Obesity is a common finding among women with PCOS. There was some difference with respect to biochemical and hormonal profile in obese and non- obese PCOS women.

P 016

Thyroid Dysfunction and Infertility

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Thyroid dysfunction has been linked to reduced fertility. Difficulty to conceive constitutes a major social and psychological burden.Thyroid dysfunction, which is quite prevalent in the population, affects many organs including female gonads, interferes with human reproductive physiology, reduces the likelihood of pregnancy and adversely affects pregnancy outcome, thus becoming relevant in the algorithm of reproductive dysfunction. Thyroid dysfunction can lead to menstrual disturbance, anovulatory cycles, and decreased fecundity. Proper management of thyroid dysfunction can result restoration of normal fertility. Therefore it is very important to screen thyroid abnormalities among women with infertility. This study aimed to determine association of thyroid dysfunction among infertile women. This study comprises total of 100 primary infertile women with age ranging from 20 to 35 years. Blood samples were collected and subjected for estimation of thyroid hormones. Out of 100 cases 547 (74.4%) were euthyroid. 18 (18%) have primary hypothyroidism, 2(2%) have primary hyperthyroidism. Awareness of the thyroid status in the infertile couple is crucial, because of its significant, frequent and often reversible or preventable effect on infertility.

P017

Study of Cardiovascular Risk Factors in PCOS

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Polycystic ovary syndrome (PCOS) is a complex metabolic, endocrine and reproductive disorder. In PCOS central adiposity, insulin resistance, dyslipidemia, diabetes and hypertension play a contributory role in increasing the cardiovascular risk profile. Some studies have found a 7 fold increase in the risk of MI in PCOS women due to this associated increased prevalence of risk factors of CVD.The aim of this study was to estimate the role of biochemical risk factors for CVD like lipid profile, insulin resistance and homocysteine levels in women with PCOS. A case control study constituting 30 diagnosed cases of PCOS, as per Rotterdam Criteria, and 30 age matched healthy controls, of age groups from 15 to 40, were selected. Biochemical markers aimed at evaluating the risk factors for CVD were measured and compared using AU 480 and ELISA assay.The mean age of cases with PCOS was 24.37±4.39 years compared to 24.9±4.8 years in controls. Women with PCOS had significantly higher levels of: fasting insulin (P=0.001), HOMA-IR (P<0.001) hyperhomocysteinemia (80% in cases vs. 33.33% in controls or P<0.001), hypercholesterolaemia ($P < 0.040$), LDL ($P = 0.018$), oxidised LDL (P<0.001), Apo-a (P=0.012) and Apo-b (P=0.005). Hence, biochemical parameters were increased in women with PCOS comparted to healthy women. These together make PCOS women more prone to increased risk of CVD at a younger age.

P018

Strong Association of IL-6 Gene with Male Infertility -Study on an Indian Populations

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It is well documentedthat many factors may impair male fertility including, endocrinedisruptors, genetic and congenital factors, post-testicular obstruction, vascular abnormalities and antispermatogenic agents. The local regulatory control is supported by a large number of cytokines, such as tumor necrosis factor -α (TNF-α) and interleukin-6 (IL-6). Aim to investigate the associations of SNPs in the candidate genes IL-6 of the infertile subjects along with assessment of hormone levels and sperm cell death in Indian population.We undertook genotyping on a total of 640 individuals, including 160 fertile donors as controls and three subgroups of infertile men, normozoospermic (idiopathic unexplained; n=160), oligozoospermic (n=160) and asthenozoospermic (n=160). These participants were selected from Departments of Biochemistry and Urology, K.G's Medical University, Lucknow, India. We used allele-specific polymerase chain reaction (PCR) and PCR-RFLP to investigate the substitution of the guanine (G)-to-cytosine (C) at position -174 in the promoter regions of the IL-6 genes and their relation to male fertility and sperm function. We found that the substitution level from G to C in the IL-6 genes was significantly higher in the patient groups as compared with the control group. Furthermore, apoptosis and necrosis levels were significantly higher in oligozoospermic and asthenozoospermic infertile subject, respectively. Also, a significant increase in the level of ROS was observed in both oligozoospermic and asthenozoospermic subjects. In contrast, a significant decrease in the levels of testosterone and luteinizing hormone was observed along with increased prolactin and follicle stimulating hormones of infertile subjects. IL-6 G-174C substitution is strongly associated with male infertility in Indian population. Allele and genotype meta-analysis also supported its strong correlation with male infertility, thus establishing it as a risk factor.

P019

Association of VDR Gene (FokI) Polymorphism & Vitamin D levels with Insulin Resistance in PCOS

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Polycystic ovary syndrome (PCOS) is the most common female endocrine disorder in women of reproductive age, presenting in up to 18 % of this population. Women with PCOS display greater prevalence of insulin resistance (IR) and compensatory hyperinsulinemia than non-affected women. Vitamin D deficiency has been suggested to be a causal factor in the pathogenesis of insulin resistance in women with PCOS. Vitamin D receptor (VDR) regulates more than 3% of the human genome, including genes that are crucial for glucose metabolism. The aim was to study VDR gene (FokI) polymorphism & 25(OH) D levels in PCOS and the relationship between VDR gene (FokI) polymorphism & 25(OH) D with markers of IR in women with PCOS.VDR gene polymorphism & 25(OH) D levels were assessed and compared between 50 diagnosed PCOS women and 50 age and BMI matched control women. VDR gene polymorphism & 25(OH) D levels were compared with various markers of IR (Fasting Plasma Glucose, Fasting S. Insulin, HOMA-IR) between the two groups. 25(OH) D levels were significantly lower in women with PCOS than control group (P = 0.033). Low 25(OH) D levels in women with PCOS were associated with higher insulin levels (r_s = -0.272, P= 0.056), higher HOMA-IR (r_s = -0.296, P = 0.037) and higher plasma glucose levels (r_s = - 0.266, P = 0.060). No significant difference was seen in genotypic distribution of C/T genotypes between cases and controls.25(OH) D levels were significantly lower in PCOS patients as compared to controls. Low 25-OH vitamin D levels were positively associated with hyperinsulinemia in women with PCOS.

P020

Effect of *vitex negundo* Aqueous extract on Induced Endometriosis in Sprague Dawley Rats

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Endometriosis is the growth of endometrial tissue in ectopic places outside the uterus. It affects 10% of women in reproductive age and is the major cause of infertility and

gynecological problems. Siddha physicians claim that *Vitex negundo* leaf decoction reduces the symptoms in clinical practice. This study was carried out to find scientific evidence for the claim. The aim was to evaluate the effect of *Vitex negundo* aqueous extract on induced endometriosis in Sprague dawleyrats. Endometriosis was surgically induced in nulliparous female Sprague dawley rats with regular estrus cycle by auto grafting endometrium tissue on peritoneum. After 30 days of implantation, rats were allocated into two groups (n=6). Control group did not receive any drug, whereas test group received an oral dose of *Vitex negundo* aqueous extract for 30 days. After 30 days of treatment, the endometriosis cyst size and adhesion were measured and blood was collected for hematology. MDA and Thiol levels were estimated in blood as well as in cyst sample. Statistical analysis was done using unpaired t test by keeping P<0.05. The test drug significantly controlled the body weight gain (P=0.03), reduced adhesionby 40% (P=0.03), and reduced cyst size by 69% (P=0.05). The test drug significantly increased thiol in blood (P=0.03), as well as in endometriosis cyst (P=0.50), and also reduced the blood MDA level (P=0.001). Our study has created the scientific evidence for the siddha physician's claim on *Vitex negundo* by demonstrating increase in antioxidant and reduces inendometriosis cyst size, adhesion and oxidant levels.

P021

Comparison of Serum Calcium and Zinc Levels in Normal Pregnancy and Pregnancy with Pre-eclampsia

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Pre-eclampsia is the most common medical complication of pregnancy associated with increased maternal and infant mortality and morbidity. Pre-eclampsia is defined as the triad of hypertension, proteinuria and oedema occurring after 20 weeks gestation in a previously normotensive woman. Lowering of serum calcium and increase in intracellular calcium causes vasoconstriction, increase in peripheral resistance and increase in blood pressure whereas lack of zinc causes increase in lipid peroxidation leading to pre-eclampsia.The present study was conducted in randomly selected 100 pregnant females. They were divided into two study groups which included 50 pregnant females with pre-eclampsia at gestation of 20 weeks or more and control group which included 50 normotensive pregnant females. Serum calcium was done by ortho-cresolphthalein complex one method and serm zinc was done by Nitro PAPS method on semi-autoanalyser. Total serum calcium levels were found to be 9.66±0.37mg/dl in normal pregnancy and 8.18±0.24mg/dl in pre-eclampsia. The difference was found to be statistically significant. Also significantly decreased (P value=0.008) serum zinc was found in normal pregnant females (114.58±123.34) in comparison to pre-eclampsia patients (67.12±8.05). Low levels of serum calcium and zinc have been found to be involved in development and pathogenesis of pre-eclampsia. Hence it is suggested that these investigations should be an integral part of the routine workup in early pregnancy for timely recognition and intervention in pre-eclampsia.

P022

A Study of Association of Serum High Sensitivity C-reactive Protein, Uric Acid, Total Protein and Albumin Concentrations with the Severity of Pre-eclampsia

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Pre-eclampsia is one of the commonest causes of high maternal and infant mortality and morbidity rates.The present study was carried out as no such study had ever been undertaken in Northeastern India. To assess the serum concentrations of hsCRP, uric acid, total protein and albumin in normotensive pregnant women,mild and severe preeclamptics and to evaluate the correlation between these markers and the severity of preeclampsia. The study comprised of 50 preeclamptics (25 mild and 25 severe) and 50 normotensive pregnant women in their 3rd trimester. Estimation of Serum hs-CRP was done by particle enhanced turbidimetric immunoassay technique, Uric Acid by Uricase, Total protein by Biuret and Albumin by BCG Method. Serum hs-CRP and Uric acid levels were significantly higher in preeclamptics as compared tonormotensive pregnant women (P<0.001) and were significantly elevated in severe than in mild preeclamptics (P<0.001). Serum albumin was significantly lower in preeclamptics than in normotensive pregnancies and was lower in severe than in mild preeclamptics (P<0.001). Serum total protein was not significantly different between pre-eclamptics and normotensive pregnant women. In preeclamptics, positive statistically significant correlation was found between hsCRP/uric acid and negative statistically significant correlation were found between hsCRP/albumin and uric acid/albumin. Serum hs-CRP and Uric Acid levels increased and Albumin levels decreased as disease progressed from mild to severe pre-eclampsia. Early assessment of serum hsCRP, Uric acid and Albumin may be used as prognostic markers and may be regarded as factors for risk stratification.

P023

Correlation of Maternal Age and Free β hCG in First Trimester of Pregnancy: Effect on Pregnancy Outcome

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Free β hCG is a glycoprotein synthesized by the syncitiotrophoblast of the placenta. It appears in maternal circulation soon after implantation and increases in concentration till 10th week. It promotes the maintenance of the corpus luteum during pregnancy which secretes the hormone progesterone during first trimester.The maternal free β hCG is correlated with amount of placental tissue and placental function. Advanced maternal age is thought to be associated with placental insufficiency.The aim was to estimate the level of free β hCG between age group of 20- 40 years during first trimester of pregnancy.To investigate the correlation of maternal age with serum free β hCG level and the correlation between maternal BMI and baby's birth weight. The study group comprises of pregnant women within the age of 20-40yrs with singleton pregnancy who came for routine first trimester screening. Maternal serum free β hCG level was measured by ECLIA. BMI was calculated using formula. Baby's birth weight was obtained from the hospital records. Maternal age inversely correlated with serum free β hCG level where as, maternal BMI correlates linearly with baby's birth weight. P value <0.001 was taken as significant.The study shows maternal age has a negative correlation with serum free β hCG level which may be due to placental insufficiency withadvanced maternal age.The study also shows maternal BMI has a positive correlation with baby's birth weight. As there are several maternal factorsrelated to infant birth weight, it is not possible to single out any particular factors affecting birth weight.

P024

A Study on Role of Catsper Gene in Male Infertility

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Male infertility accounts for ~30 to 50% of all infertility cases, with unexplained infertility accounting for a further 10 to 20% of cases. For fertilization, there are many obstacles a sperm has to overcome and Ca^{2+} ions are involved in nearly every step and therefore continue to loom as the primary determinant of sperm cell behaviour. Cation channels of sperm is a small family of ion channels, normally referred to as Catsper channels (CatSper1, 2, 3 & 4) which are putative six-transmembrane (6TM) spanning proteins and seem to be specific to sperm cells. CatSpergeneis located on chromosome 15 q13-q15 and is involved in sperm motility and hyperactivation.We designed our study with an aim to investigate the CatSper2 and 4 genes mutation in all infertility cases, and analysed their correlation with clinico-pathological findings.We performed mutational analysis of the CatSper2 and CatSper4 genes in 120 infertile males and 120 healthy controls. To screen mutations in CatSper2 and CatSper4 genes, we performed PCR followed by direct DNAsequencing. On analysis of mutational screening of CatSper2 gene, we found a single nucleotide change (TGG→**G**GG) in twenty two infertile males. These are missense mutations as the changes lead to amino acid sequence change from tryptophan to glycine. We found missense and silent mutations in CatSper4 gene of 14 infertile males. On analysis of mutational screening of CatSper2 and 4 genes, there wasmissense and silent mutation that leads to conformational structural change. This could affect the normal influx and efflux of calcium ions which in turn hinders acrosome reaction during fertilisation.

P025

Association of GABRA6 Gene and Synapsin II Gene Polymorphisms with Genetic Generalized Epilepsy

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The genetic generalized epilepsies (GGEs) accounting for approximately 30% of all epilepsy cases with no underlying structural or brain lesions and are neurologically normal. GABA has been reported to be an inhibitory neurotransmitter and its effects are mediated through $GABA_A$ receptor. Synapsins are a family of synaptic vesicle associated phosphoproteins, which have been associated with the cytoplasmic surface of synaptic vesicles in presynaptic nerve terminal that regulates neurotransmitter release in mature synapses.The present study was aimed to investigate the association of $GABA_A$ α-6 subunit 1519 T>C gene and Synapsin II gene polymorphisms with GGE in a South Indian population from Andhra Pradesh. Three hundred and ten patients (male: female=203:107) with GGE were recruited along with 310 healthy

controls. Genotyping was performed by PCR-RFLP protocols. We found statistically significant difference in the genotypic distribution [(CC *vs.* TT, $\chi^2 = 26.0$; P<0.01; Odds ratio=3.6 (95%CI; 2.1-5.9)]as well as allelic frequency [(C *vs.* T; $\chi^2 =24.7$; P<0.01; Odds ratio=1.7 (95%CI; 1.4-2.2)] of GABRA6 gene polymorphism between patients and controls. Similarly, statistically significant difference in the genotypic distribution (GG *vs* AA, $\chi^2 = 64.52$; P<0.01; Odds ratio=7.3 (95%CI; 4.4-12.3) and frequency of A and G alleles [(G *vs.* A; $\chi^2 =65.78$; P<0.01; Odds ratio=2.5 (95%CI; 2.0-3.2) of Synapsin II gene polymorphism between patients and controlswere observed. In conclusion, our results suggest that the GABRA6 T>C and Syn II A>G gene polymorphisms are significantly associated with GGE in a South Indian population from Andhra Pradesh.

P026

Early Neurological Marker in Perinatal Asphyxia and its Correlation with Different Stages of HIE

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Perinatal asphyxia is a major cause of neurologic morbidity in infants. Hypoxic ischemic encephalopathy (HIE) after perinatal asphyxia is a condition in which serum concentration of brain specific biochemical markers may be elevated. Neuroprotective interventions in asphyxiated newborns require early indicators of brain damage to initiate therapy. There are very few studies about its usefulness in asphyxiated newborns. Aim of the present study was to determine the serum levels of Interleukin-6 in newborns with perinatal asphyxia and its relation with different stages of hypoxic ischemic encephalopathy.We have measured the serum values of IL-6 by ELISA method in 100 asphyxiated newborns and 100 healthy newborns (control group). Blood samples were taken on day 1 and day 3 of life in all newborns.The mean serum values of IL-6 were found to be decreased on day 3 in asphyxiated neonates and a negative correlation was seen between day 1 and 3 for IL-6. The mean values of IL-6 were decreased in different stages of HIE on day 3 as compared to day 1 and a Negative correlation was observed between day 1 and day 3 for IL6 in no HIE, HIE I, HIE II & HIE III stages. We conclude that serum IL-6 concentrations increased considerably after birth asphyxia, and the increase is associated with the severity of HIE with a poorer outcome.

P027

Telomerase Activity in Gall Bladder Cancer - In Association with p53 Proteins and CA 19-9

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Very little information is available about telomerase activity in gall bladder cancer, hence this study was undertaken to evaluate the role of telomerase in differentiating benign from malignant gall bladder disease, in association with p53 proteins, CA 19-9, CEA and total serum bilirubin. Surgical specimens and blood samples were collected from 37 patients with gall bladder cancer undergoing planned surgery at the Tata Memorial Hospital, Mumbai. Biopsy tissues and blood samples from 50 patients undergoing gall bladder surgery for a benign condition (chronic cholecystitis) were collected from the KEM Hospital, Mumbai and were stored at -80°C until the test was performed. Telo TAGGG Telomerase PCR ELISA and Anti-p53 ELISA kits were used. CA 19-9 and CEA were estimated using chemiluminescent microparticle immunoassay technology. Total serum bilirubin was estimated using 3,5- dichlorophenyldiazonium tetrafluoroborate (DPD) method. 34 of 37 specimens had detectable relative telomerase activity. 32 of 37 samples showed anti-p53 antibodies above the cut-off value. CA 19-9 was elevated in 32 of 37 samples. CEA was elevated in 21 of 37 samples. Concentration of total serum bilirubin was above the cut-off value in 13 of 37 samples. Spearman's correlation coefficient between telomerase activity and CA 19-9 level was 0.290, which is significant at 5% and between telomerase activity and p53 proteins was 0.262, which is also significant at 5%. p53 proteins and telomerase activity showed highest sensitivity and specificity as compared to CEA and CA 19-9. Spearman's correlation coefficient between telomerase activity and p53 proteins was 0.262, which is significant at 5%.

P028

Non-enzymatic Antioxidant Profile in Oral Cancer Patients

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Epidemiological studies suggest that antioxidants &/ micronutrient deficiencies play a major role in progression of oral cancer. In regards to this oxidative processes contribute in promoting stages of carcinogenesis. Though several studies have been made which attempt to evaluate levels of MDA, antioxidant vitamins &/ micronutrient status in oral cancer patients, only a few

are available w r t different grades. This aspect is studied in present work. The possible role of non-enzymatic antioxidants &/ micronutrients in prediction & prevention of oral cancer was elucidated. Eligible cases were 50 healthy subjects & 100 histopathologically-diagnosed cases of oral carcinoma (grade I & grade II) with a known history of tobacco-chewing habit in patients. Serum MDA, vitamin A (β carotenes), E, C, iron, & TIBC levels were estimated by colorimetric methods respectively. Significantly increased MDA & diminished antioxidant as well as micronutrient status was demonstrated in oral cancer patients & w r t grades, as compared to control group. Tobacco, particularly, gutkha chewers, had significantly low levels of antioxidant vitamins & micronutrient iron with elevated MDA levels as compared to pan chewers &w r t control group. Risk of oral cancer estimated was 3.5, 5.80, 11.1, & 1.71 times more in patients with low vitamin A, E, C [P = 0.001], & iron levels [P = 0.05]. An increased MDA, deficient antioxidant vitamins as well as iron levels, & long-term inflammation caused by smokeless tobacco, particularly gutkha, may be regarded as risk factors for oral cancer. The low antioxidant &/ micronutrient levels could be either a cause or effect of oral cancer or due to tobacco-chewing habit. OSCC was found to have association with increased MDA, low serum antioxidant &/ micronutrient profile of vitamin A, E, C, & iron.

P029

5-Hydroxyindoleacetic Acid and Serotonin in 24 Hours Urine of Patients with Neuroendocrine Tumors

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Carcinoid tumors are neuroendocrine neoplasms, primarily of the gastrointestinal tract. There are three main areas of origin for carcinoid tumors: foregut, midgut and hindgut. They synthesize, store and release substances including serotonin, histamine, prostanglandins, kallikrein, bradykinins, substance P, gastrin, corticotrophin and neuron-specific enolase. Carcinoid tumors convert most of the tryptophan to serotonin and to 5-Hydroxyindoleacetic acid (5-HIAA). The objective of the study was to see the usefulness of 24 hours urinary 5-HIAA and serotonin in locating the site of the neuroendocrine tumor (NET).15 benign subjects and 172 cases with neuroendocrine tumors (134 patients of foregut, 23 of midgut and 15 of hindgut tumors) of the age group of 14- 86 years having histological evidence of malignancy were included in the study. 24 hours urine collection was done with prior appointment and instruction. 5-HIAA & serotonin levels were estimated by column chromatography using kits from Biosystem and quantified spectrophotometrically. Internal and external quality control samples were run during the analysis to check precision and accuracy of the estimation. Students't' test for unpaired number was used for statistical analysis after confirming homogeneity of variances. On comparing the mean levels of urinary 5-HIAA and serotonin of benign tumors and patients with foregut, midgut and hindgut neuroendocrine tumors, it is found that the mean levels of 5-HIAA and serotonin were significantly raised in all. The levels of 5HIAA in foregut NET were increased by 2.15 times compared to serotonin whereas the pattern was exactly the reverse in midgut where serotonin was raised by 3.29 times compared to 5HIAA. The levels of 5HIAA and serotonin in hindgut NET were almost the same. Tumors from midgut cells, contain and release large quantities of serotonin. These amounts may not be fully reflected in the amount of 5-HIAA in urine, because little is metabolized. Tumors derived from foregut cells produce large amounts of serotonin, which is oxidized within the tumor to 5-HIAA. Tumors derived from hindgut cells rarely produce excess serotonin or 5-HIAA. The study reveals that 24 hours urinary 5HIAA in conjunction with serotonin is a better indicator of the foregut, midgut and hindgut NETs.

P030

Usefulness of Pancreatic Enzymes and Tumor Markers CA19-9, CEA in Cancer of Pancreas, Gallbladder and Gallbladder with Liver Metastasis.

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Gallbladder carcinoma is the most common biliary tract cancer, accounting for 3 % of all tumors. The most useful and widely investigated marker for colorectal cancer is CEA, and for pancreatic adenocarcinoma is CA19-9. Increased concentrations of CA19-9 are however, not specific for adenocarcinoma of the pancreas. High levels can also be found in other GI tract malignancies especially with advanced disease. The objective was to determine the usefulness of CA 19-9, CEA and pancreatic enzymes in cancer of pancreas, gallbladder and gallbladder with liver metastasis. 51 normal healthy controls, 55 patients having carcinoma of pancreas, 73 patients with cancer of gallbladder and 58 patients with cancer of gallbladder with liver metastasis. Age group of all cases studied belongs to 14-84 years. Total amylase, p-amylase, Lipase were estimatedusing Roche kits on fully automated Beckman Coulter AU640 chemistry analyzer and CEA and CA 19-9 assays were carried out on fully automated immunoassay analyzer Architect i2000sr system. Student's 't' test for unpaired number was used for statistical analysis after confirming homogeneity of variances. The mean levels of total amylase, pancreatic amylase, lipase, CEA and CA19.9 were significantly elevated in cancer of pancreas compared to normal control (P<0.001). In pancreatic cancer levels of salivary amylase were lowered (P<0.01). Cancer of gallbladder patients

shows no significant changes in the levels of total amylase, pancreatic amylase, salivary amylase and lipase but significantly increased levels of CA19.9 and CEA (P<0.001). Cancer of gallbladder with liver metastasis patients shows no significant changes in the levels of pancreatic amylase and lipase but showed decreased levels of total amylase (P<0.02) and salivary amylase (P< 0.01). CEA and CA19.9 were significantly elevated (P<0.001). Our studies indicate that measurement of pancreatic enzymes and tumor markers CEA and CA19-9 were useful to differentiate cancer ofpancreas, gallbladder and gallbladder with liver metastasis.

P031

Association of Plasma D-dimer levels with Histologic Subtypes and Grades of Lung Cancer

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Activation of coagulation and fibrinolysis is frequently encountered among cancer patients. Such tumors are supposed to be associated with higher risk of metastases and eventually worse outcome. An elevated plasma D-dimer level, indicates the activation of coagulation and fibrinolysis. The study was aimed to investigate the association of plasma D-dimer levels with Histologic types and Grades of newly diagnosed lung cancer patients. Plasma D-dimer levels were measured in 30 newly diagnosed lung cancer patients and 30 healthy subjects as control group using an enzyme immunoassay kit. The patients had no other history of coagulation system disorders or anticoagulant therapy. Prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratio (INR), hemoglobin (Hb), platelets (Plt) and white blood cells (WBC) count were also measured before chemotherapy. The median age of the patients (27 males/3 females) was 50 years (range 25-75yrs). Plasma D-dimer levels were evaluated according to histopathological types and grades of disease. Histologic subtypes were non-small cell carcinoma (90%) and small cell carcinoma (10%). The mean D-dimer level of the patients was 2.620 µg/dl, which was higher than that of the control group (0.289). The D-dimer, PT and INR levels of the patients with lung cancer were significantly higher than in the control group (P=0.001). The D-dimer level was also found to be significantly higher in metastasized lung cancer. The results suggest that pre-treatment plasma D-dimer levels increases with the grades and metastasizing lung cancer. However, further prospective studies are needed in a larger population to confirm these findings and to use it for predicting the clinical outcome in patients with lung cancer.

P032

CA-125 Just a Tumour Marker : A Misnomer

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Cancer antigen-125 (CA-125) is a high molecular weight glycoprotein used as a marker for ovarian carcinoma. But its involvement is also observed in many benign conditions particularly in liver cirrhosis and ascites. A total of 30 patients with chronic liver disease (liver cirrhosis), 20 patients with acute liver disease were enrolled in this study. The degree of ascites was graded to into mild, moderate and severe. CA-125 levels were evaluated through Enzyme -Linked Immunofluorescence Assay (ELIFA) and were compared with other liver parameters and USG findings of all the patients. 30 CLD patients with ascites had CA-125 levels significantly elevated and it was related with the grades of ascites, whereas (18/20) patients without ascites had CA-125 levels under the normal range. However, the levels of CA-125 were significantly higher in patients with ascites compared to patients without ascites. The elevation of CA-125 in liver cirrhosis is related to the amount of ascites. Therefore, CA-125 may be used as a predictor of ascites in patients with liver cirrhosis.

P033

Interplay between *MDM2* and *p53* Polymorphisms has Significant Clinical Role in Oral Cancer

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Polymorphic variants of *MDM2* and *p53* might have effects on p53 responses in oral cancer and thuson outcome of oral cancer.Objective is to evaluate the role of *MDM2* polymorphism (T>G, rs2279744) and its association with *p53* Arg72Pro polymorphismin oral cancer. 236 cases and 212 controls were enrolled for the study. Genotypes of *MDM2*and *p53* polymorphisms were determined by PCR-RFLP method. TT genotype of *MDM2* in combination with Arg/Arg genotype of *p53* revealed decreased risk of oral cancer. All the genotypes of *MDM2* in combination with Pro/Pro genotypes of *p53*exhibited significant high risk to progress towards advanced stage. Recurrence rate was higher in patients having T allele. Further, recurrence rate was significantly higher in patients having advanced stage of the disease and T allele. Risk of recurrence was significantly higher in patients having advanced stage of the disease and T allele in combination with

Arg/Arg genotype of *p53*. GG genotype was associated with higher disease free and overall survival. GG genotype was associated with higher disease free and overall survival in patients having Arg/Arg genotype of *p53*. However, GG genotype was associated with lower disease free and overall survival in patients having Pro/Pro genotype of *p53*. Moreover, GG genotype was also associated with significantly higher disease free and overall survival in advanced stage patients. *MDM2* polymorphism alone as well as in combination with *p53*Arg72 Pro polymorphism could modulates the outcome of oral cancer patients.

P034

p53 Mutation Spectrum and its Role in Prognosis of Oral Cancer Patients from Gujarat, West India

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p53 mutations play a vital role in etiopathogenesis of oral cancer. They show variations in type, codon specificity, exposure to specific tobacco carcinogens which in turn show geographical variations. We aimed to analyze the frequency of *p53* mutations in adjacent normal and malignant tissues in oral cancer patients and their effect on clinicopathological features, local recurrence and survival. Aim was analyze the frequency of *p53* mutations in adjacent normal and malignant tissues in oral cancer patients and their effect on clinicopathological features, local recurrence and survival. *p53* mutation analysis was performed on 46 paired tissue samples (adjacent normal and malignant) using PCR-SSCP and sequencing. Sequencing confirmed 51 *p53* mutations with three distinct novel mutations (frameshift deletion in exon 4; G>T transversion at codon 117 in exon 4 and G>A transition at codon 319 in exon 9). Distinct pattern of *p53* mutations was observed: more common T>C transitions, recurring mutations at codon 90, 116 (exon 4). No significant association with clinico-pathological variables. Early stage tumors with *p53* mutations had significant higher chances of recurrence. Probability of developing local recurrence was higher in cases with *p53* mutations both in adjacent normal and malignant tissues. Significant low disease free and overall survival was observed in cases harboring truncating and transcriptionally non-active mutations.Very high frequency and diverse pattern of *p53* point and frameshift mutations was observed. 3 distinct novel mutations in exon 4 and 9. Most interesting finding is: early stage tumors with mutations were at high risk of developing recurrence. Analyzing the type of *p53* mutation could be important prognostic factor in oral cancer.

P035

Role of CA 15.3 with and without Metastasis in Breast Cancer Patients

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Cancer Antigen 15-3 (CA 15-3) is a tumor-associated antigen used as serum marker for breast cancer surveillance in patients and for monitoring the response to treatment. The specificity and sensitivity of a serum tumor marker are important in establishing its potential clinical utility for a specific type of neoplasm. The present study was aimed to assess the level of CA 15-3 in without metastasis and with metastasis of breast cancer patients compared with control group. The total number of 110 samples were categorized into 3 groups such as Group I healthy individual (n=30), Group II without metastasis (n=30) and Group III with metastasis Breast Cancer patients. All participated provided informed consent with blood sample for the test of CA 15.3. Blood sample was centrifuged and serum was separated and used for the estimation of ELISA test for CA 15.3. All the results were analyzed by using SPSS 16.2 version. A P value < 0.05 is considered to be statistically significant.The serum level of Breast cancer patients significantly increase in the without metastasis (16.80±3.5) compared with control group (3.4±1.1) and highly significant was found in the metastasis Breast Cancer patients (80.2±11.2). CA 15.3 has been attributed as breast cancer serum tumor marker. It is a significantly more powerful marker for determining response to treatment. Elevated level of without and with metastasis of CA 15-3 significant prognostic indicator and a useful factor for cancer progression in Breast Cancer patients.

P036

Usefulness of CA125 and TPS in Diagnosis and Treatment of Ovarian Cancer

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This study was undertaken on 43 patients of Ovarian Cancer and 33 patients of benign Ovarian Tumors. Serum levels of TPS and CA125 were determined in all cases before and after surgery. Before surgery, the sensitivity, specificity, PPV, NPV for CA125 were 90.7%, 72.73%, 81.25% and 85.25% respectively and those obtained for TPS were 90.7%, 66.67%, 78% and 84.62%

respectively. When both the tests were used in combination, the values obtained were 100%, 54.55%, 74.14% and 100% respectively. After radical surgery TPS and CA125 serum levels remained elevated in 37% and 85% of cases respectively demonstrating a faster decrease in post-surgical TPS levels. The above observations are valuable as they demonstrate that parallel use of CA125 and TPS in Ovarian cancer patients increase the sensitivity and treatment outcome in radical surgery.

P037

Assessment of Pre and Post Treatment Status of Serum Lactate Dehydrogenase and Alkaline Phosphatase in Lung Cancer

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The most prominent biochemical feature of malignant cells remains the high glycolytic activity in production of large quantity of lactic acid. Serum LDH is the consequence of the disruption of dividing malignant cells whose metabolic hallmark is anaerobic glycolysis that leads to increase LDH activity. Its increase reflects tumour mass and response to therapy. Serum levels of alkaline phosphatase (ALP) have been used in the clinical evaluation of numerous diseases, including malignancies. Objective was to estimate serum LDH and ALP in lung cancer patients before and after treatment, to see any changes and then to find out any correlation between serum LDH and ALP levels and stages of lung cancer. Fifty freshly diagnosed lung cancer patients of different stages were taken as study group and a total of 30 normal healthy individuals were taken as control group. Blood samples were collected before any treatment was given then follow up second samples were collected after the completion of treatment. Serum LDH and ALP were measured by colorimetric method.The pre-treatment mean serum LDH (439.42±145.31) and serum ALP (299.60±144.29) was significantly higher than mean serum LDH (221.06±34.00) and ALP (162.30±20.61) in the control group. The serum LDH and ALP decreased significantly after treatment and the difference in mean of these enzymes level was statistically significant ($P<0.01$). The pretreatment serum LDH and ALP had shown a positive correlation with stages of lung cancer. Serum LDH and ALP level might be a valuable biochemical index in the diagnosis and prognosis of lung cancer and as biomarker of disease progression and in monitoring response to therapy.

P038

Biomarkers for Early Detection of Ovarian Cancer: Beyond CA125

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Ovarian cancer is the most fatal type of gynecologic malignancy, afflicting women in post menopause, with more than 70% diagnosed at advanced stage. In 2014, estimated cases of death due to ovarian cancer will be 64.9% out of number of patients diagnosed with ovarian cancer in United States alone (SEER, NIH) due to poor prognosis rate at early stage of disease. In most cases at an early stage (IA, IB, IC) women remain asymptomatic with only 20% of patients are diagnosed at stage I. Patients diagnosed at advanced stage have poor survival percentage (20% to 40%) as compared to patients diagnosed at early stage (90%). It still remains challenging to detect recurrences of ovarian cancer as early as possible post treatment stage. Novel biomarkers identified so far to diagnose ovarian cancer include CA125, CA72-4, CA15-3, MCSF, LASA, SMRP, HE4, activin, inhibin, osteopontin, epidermal growth factor (EGFR), ERBB2 (Her2), KLK6/7, GSTT1, PRSS8, FOLR1, ALDH1, MicroRNA signatures, ctap3 proteins. Objective of study novel biomarkers reported by various researchers for diagnosis and monitoring ovarian cancers. As compared to CA125, other markers especially HE4 found to be more specific and combination approaches of different markers reported higher detection rate. MicroRNA signatures reported to screen asymptomatic populations potentially. Ctap3 found to distinguish between Ovarian and non ovarian cancer. Novel biomarkers serve as more effective screening modality for ovarian cancer than conventionally used biomarker CA125, thus enhancing treatment procedures for this lethal disease.

P039

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 are having 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 & 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 was significantly low as compared to benign groups and healthy controls

P040

Demographic Profile and the Diagnostic Validity of C-reactive Protein for Discrimination of Malignant Type in Patients with Exudative Pleural Effusion

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Clinical history and physical examination are helpful in indicating the potential causes of pleural effusions (PEs). However, the accurate diagnosis and establishment of the causes of PE is an ongoing challenge in daily clinical practice. Test able to help in the diagnostic work-up of pleural exudates are needed. C-reactive protein (CRP) may be useful for distinguishing between benign and malignant exudates. Objective is to assess demographic profile and determine the validity of pleural fluid CRP concentrations for differentiating pleural effusion of malignant from non-malignant etiology. In this hospital based observational study, outcomes were assessed in 187 cases with exudative PE, using an immunoturbidimetric method (Transasia EM360 autoanalyser). Significance of difference in means in groups was inferred by ANOVA and Post HOC test. Commonest type of exudates was tuberculer (54.6%)followed by malignant (31.02%),chronic non specific inflammation (5.9%), parapneumonic (4.8%). Statistically significant difference were observed in mean age, sex, history of smoking, tuberculin test and pleural fluid CRP, ADA, Protein. Mean ADA was significantly higher in Tuberculer type while mean CRP was significantly higher in parapneumonic than other types (P<0.001). In addition, a optimum cut-off point through ROC was observed below 52 mg/ l for malignancy with 69% sensitivity of CRP and specificity of 86%. In differential diagnosis of pleural effusion, pleural fluid CRP may prove rapid and practical method of differentiating malignant from non-malignant pleural effusion.

P041

Multiple Myeloma and Laboratory Investigations – The CMC Vellore Perspective

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Multiple myeloma is a malignant tumour of the B cell Linkage characterised by excessive proliferation of abnormal plasma cells, secreting large amounts of abnormal immunoglobulin molecules known as paraproteins which can be detected by the presence of M-protein in serum and/ or urine electrophoresis. Objective is to investigate the prevalence of patients biochemically diagnosed with monoclonal gammapathy by our laboratory and the usefulness of each test audited in arriving at the diagnosis. The biochemical investigations audited in this study were : total protein and albumin, serum protein electrophoresis by automated Minicap system, urine BJP and serum immunofixation by agarose gel electrophoresis, serum free light chains (kappa and lambda) and serum immunoglobuins by turbidometer. Over a 6 month period there were 2100 serum samples received for estimation of serum protein electrophoresis for suspected cases of myeloma , out of which 420 (20%) showed positive for myeloma or BMGUS. Of these 420, some had M-band positive and /or BJP positive. These were from patients of two categories, new (180) and follow up (240). Out of the 420 positive cases, there were 14% cases with M band > 3 g%, 60% with M band <3 g%, Biclonal gamopathy 6%, faint bands 14%, and beta -2 overlap 7%. IFE and serum free light chains were carried out only on samples from patients requested by the physician of our hospital. Clinical laboratories play an important role in confirming the biochemical diagnosis of gammopathies. Hence the suggested strategy for investigations of suspected cases of myeloma is to use serum total protein and albumin, serum protein electrophoresis and urine protein electrophoresis as the front line tests. When a M- band is present or a BJP band is seen on the urine electrophoresis, then serum immunoglobulins, IFE and serum free light chains should be done in all cases as second line tests.

P 042

Single Nucleotide Polymorphisms (SNPs) of Folate Metabolizing Enzymes and their Association with Brain Tumors

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The genes of folate metabolizing enzymes display SNP variations. SNP at C677T and A1298C of MTHFR (Methylenetetrahydrofolate reductase), A2756G of MTR (Methionine synthase) and A66G of MTRR (Methionine synthase reductase) genes have been implicated in the development of various tumors. We studied these SNP variations for their association with brain tumors in Indian population.We aimed to determine the allelic and genotypic frequencies of SNPs in MTHFR (C677T and A1298C), MTR (A2756G) and MTRR (A66G) genes and their association with glioma and meningioma in Indian patients as compared to healthy control subjects. Polymorphism analysis of MTHFR (C677T) and MTR (A2756G) genes was done by PCR-RFLP (PCR-Restriction Fragment Length Polymorphism) while MTHFR (A1298C) and MTRR (A66G) gene polymorphisms were analyzed by ARMS (Amplification Refractory Mutation system) PCR using genomic DNA of 67 control subjects, 38 glioma patients and 38 meningioma patients.We observed no significant differences in the allelic and genotypic distribution of SNPs in MTHFR (C677T and A1298C), MTR (A2756G) and MTRR (A66G) genes in patient (glioma and meningioma) groups with respect to controls. Preliminary results did not show any significant association of the SNPs in genes encoding folate metabolizing enzymes with meningioma or glioma. Further analysis with an increased number of samples is ongoing.

P043

Evaluation of Serum Butyrylcholinesterase and Nitric Oxide level in Breast Cancer Patients

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Breast cancer is the most common cancer in women all over India and accounts for 25 to 31% of all cancer in women. Currently many markers are available for screening of this cancer which include cancer antigen 15-3, Cancer antigen 27.29 and carcinoembryonic antigen. But these markers are also elevated in conditions other than cancer. In this regard, there is a need to develop a biomarker which can indicate the occurrence of cancer and its prognosis. So we estimated serum butyrylcholinesterase (BChE) and nitric oxide (NO) in breast cancer patients. Objective was measure and compare Serum BChE and NO levels in female breast cancer patients and age matched healthy controls and correlate serum levels of these markers with different stages of cancer. Ethics clearance was taken from Institutional ethics committee. Fifty histopathologically proven female breast cancer patient and 50 age and sex matched healthy controls were enrolled in this study after taking consent. Serum BChE was measured by modified Ellman's method serum NO was estimated by cadmium reduction method. We found a significant increase ($P =\leq 0.001$) in serum BChE and serum NO levels in breast cancer patients compared to healthy controls. A significant positive correlation was found between serum BChE and stage of cancer. No correlation was found with levels of nitric oxide with stage of cancer. Serum BChE and nitric oxide may have a role in pathogenesis of breast cancer.

P044

A Study on Serum Total and Free PSA Levels in Breast Cancer

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Breast cancer (BC) is one of the most common malignancies in women and a major cause of death worldwide. Besides the conventional tumour markers, search for newer ones has been going on that may contribute to improved diagnosis and treatment. It is widely accepted now a days that PSA is not prostate specific and that it has wide spread distribution. Objective was to measure the relative proportion of molecular forms of PSA in BC both before and after surgery and to analyze their association with the severity of the disease process. Place of study is Dept. of Biochemistry, GMCH & BBCI GHY; Cases are 50 newly diagnosed patients of BC; Control:50 healthy subject. Total & free PSA was measured by DS-EIA-PSA kit using ELISA. Lipid profile and fasting blood sugar was done in automated analyzer. $P<0.05$ was considered significant.A fall in postoperative value of total & free PSA in BC was noticed. In grade I tumours the mean value of total and free PSA were higher than those with grade III tumours. Mean value of fasting blood sugar,total cholesterol and LDL in BC was higher than the control group.The present study was undertaken, keeping in mind the growing incidence of breast cancer cases in our society and with a view to establish PSA as a possible new marker for breast cancer.

P045

Estrogen and Progesterone Levels in Post Menopausal Breast Cancer Patients

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Breast cancer is a malignant proliferation of epithelial cells lining the ducts or lobules of the breast.Worldwide, breast cancer is the most common invasive cancer in women. Breast cancer comprises 22.9% of invasive cancers in women and 16% of all female cancers. Estrogens are considered to play a major role in promoting the proliferation of both the normal and neoplastic breast epithelium. Their role as breast carcinogens has recently confirmed by epidemiological studies.Progesterone also contribute to breast cancer biology. Aim was compare the levels of estrogen and progesterone in post menopausal breast cancer patient with age matched healthy controls.This study was conducted in the Department of Biochemistry in collaboration with the Department of Oncology, PGIMS, Rohtak. The study group comprised of 25 female patients with breast cancer confirmed by biopsy and 25 age matched healthy females with postmenopausal status were taken as control group. Serum estrogen and progesterone levels were estimated in study and control group. Serum estrogen and serum progesterone were estimated by competitive immunoassay using direct chemiluminiscence technique on ADVIA-CENTAUR CP.The mean levels of estrogen and progesterone were significantly higher (71.56±68.13 ng/mL; $P \leq 0.001$ and 4.32±4.19 ng/mL; $P<0.05$ respectively) in cases than controls (1.38±1.04 ng/mL and 3.14±2.65 ng/mL respectively). Our results indicate that estrogen and progesterone should be routinely estimated in breast cancer patients for better treatment approaches as well as monitoring of disease progression since estrogen progesterone receptors are correlated with severity and response to treatment in certain studies.

P046

Relationship of Serum Lactate Dehydrogenase and Alkaline Phosphatase with Lung Cancer

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Enzymes can be used as diagnostic and prognostic markers in lung cancer patients for detection, monitoring and evaluation of treatment. Objective was to analyze the level of serum Lactate dehydrogenase (LDH), and Alkaline phosphatase (ALP) activity in lung cancer patients receiving treatment. A total of 50 lung cancer patients suffering from different stages of the disease were selected for the study. All patients were freshly diagnosed and clinically staged. A total of 30 age, sex matched apparently healthy individuals were taken as the "control group". Study result reveals that mean serum LDH (439.42±145.31 IU/l) and ALP (299.60±144.29 U/l) are significantly higher in patients with lung cancer as compared with control group (LDH 221.06±34.00 IU/l and ALP 162.30±20.61 U/l). The difference is statistically significant. Serum levels of LDH and ALP are also seen to rise with stages of disease showing positive correlation. The serum levels of LDH and ALP are also seen to reduce in response to therapy with tumor mass regression, which was more pronounced with serum LDH. Measurement of serum LDH and ALP activity can be useful marker in lung cancer diagnosis, in monitoring disease progression and evaluating response to therapy.

P047

Clinical Status v/s Tumor Markers Levels in Esophagus Cancer Patients Following Therapy

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Tumor markers are used for follow up of treatment, either for patient based or experimental conditions. There has been much optimism that sequential estimation of tumor markers concentration during the follow up of cancer patients who have undergone curative resection or therapy might detect occult recurrent disease early enough for further successful treatment. Tumor markers levels were studied in esophagus cancer patients before and after therapy to evaluate their prognostic significance. Results were compared with clinical response and imaging studies. Blood samples were collected from 50 patients of esophagus cancer before and six months after therapy and analysed for CEA, AFP, β-HCG by Chemiluminiscence immunoassay (CLIA). CA19-9 was analysed by Enzyme linked immunosorbent assay (ELISA). Statistical differences were estimated by 't' test.Mean serum level of tumor markers (except AFP) were significantly higher in progressive disease CEA ($t=2.004$, $P<0.05$), CA19-9 ($t=2.031$, $P<0.05$), AFP ($t=0.850$, $P>0.05$) β-HCG ($t=0.043$, $P<0.05$), and were significantly lower (except AFP with no change) in improved condition CEA ($t=3.069$, $p<0.01$) CA19-9 ($t=2.958$, $P<0.01$) AFP ($t=1.761$, $P>0.05$) and β-HCG ($t=3.264$, $P<0.01$) when compared with levels before therapy. There was no change seen in tumor markers level in patients who were in stable condition. The results correlated well with imaging studies and clinical response of patients. By using the information that these markers can provide, patient-specific treatment protocols

can be developed, implemented, and monitored for improved patient outcomes.

P048

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

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As oral squamous cell carcinoma (OSCC) is one of the most common malignancies recognized nowadays, its early detection helps to provide a good quality of life for the patients. Studies have correlated cholinesterase with tumorigenesis, cell proliferation and cell differentiation. Butyrylcholinesterase has been used as a biochemical marker in the management of cervical cancer. Objective was to estimate serum butyrylcholinesterase levels in biopsy proven oral cancer patients before radiotherapy and compare it with that of respective post radiotherapy levels. 20 healthy controls and 39 OSCC patients (stage II n=8, stage III n=10, stage IV n=21) were included in the study. In cases, blood samples were collected before and after radiotherapy. The activities of butyrylcholinesterase were estimated. Serum butyrylcholinesterase levels were significantly elevated ($P< 0.0001$) in oral cancer patients as compared to that of controls. In oral cancer patients, there was a significant decrease in butyrylcholinesterase levels ($P= 0.005$) after radiotherapy irrespective of stage of cancer as compared respective pre radiotherapy levels. Radiotherapy decreased serum BChE levels of cancer patients significantly irrespective of stages as compared to their respective preradiotherapy levels. This implies a possible role for this parameter in prognosis of oral cancer patients

P049

Assessment of Correlation between free Prostate Specific Antigen and Gonadal Steroid Hormones in Female Breast Cancer Patients

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Worldwide breast cancer is the most common cancer in women comprising of 22.9% of invasive cancers. Prostate specific antigen (PSA) is produced from female breast. Free PSA (fPSA) increases significantly in women with invasive breast cancers and its level decreases following surgery. PSA expression in epithelial cells is regulated by gonadal steroids significantly. Our aim was to measure the serum fPSA levels in breast carcinoma patients both pre surgically and post surgically and its association with serum levels of progesterone and β estradiol. The study was conducted in CNMC, Kolkata, West Bengal in the Departments of Biochemistry and General Surgery after obtaining ethical committee permission. Blood was collected aseptically from 44 histologically proved female breast carcinoma patients pre-surgically. fPSA, β estradiol and progesterone was measured. Same parameters were again measured post surgically 10 to 14 days later. Blood from 40 control subjects was also collected and treated similarly. With post hoc analysis showed a significant reduction in fPSA level in post surgical cases. Estrogen and progesterone although showed significant positive correlation between them failed to show any such correlation with fPSA. Malignant breast tissues are significant sources of fPSA which does not depend on the gonodal steroids. We conclude that fPSA level can be monitored for evaluating progression of the disease along with remedial effects of successful management.

P050

Radioprotective effect of *O.sanctum* on the Salivary Glands of Rabbits Exposed to High Dose of ^{131}I

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Oral administration of ^{131}I to the patients of differentiated thyroid cancer is routinely used to ablate remnant thyroid tissue or metastasis.Though internal administration of ^{131}I is considered to be safe, it has been found to be associated with salivary gland damage. Around 30% of the patients exhibit permanent salivary gland damage resulting in xerostomia affecting their quality of life. At present amifostine is the only standard FDA approved radioprotectant available in the market for controlling the salivary gland damage but its associated with side effects such as hypotension and allergic reactions. Objective was to study the radioprotective properties of *O. sanctum* to the salivary glands of subjects exposed to high dose of ^{131}I. The study was carried out using New- Zealand White rabbits. They were divided in 4 groups as follows, Gr I: Control (n=2), Gr II: Rabbits (n=3) exposed to ^{131}I (1GBq), Gr III: Rabbits(n=3) were pre supplemented with *O.sanctum* (40mg/Kg bw) for 5 days and subsequently exposed to ^{131}I (1GBq). Gr IV: Rabbits (n=3) were pre supplemented with amifostine (200mg/kg bw) i.v 30 min before the 131I exposure (1GBq). Amifostine was used as a positive control. The animals

after exposure to compounds and/or ^{131}I were housed in RMC Animal house for 6 months and at intervals salivary amylase, protein, serum SGOT, serum SGPT, hematological parameters were studied. At the end of six months uptake of ^{99m}Technetium pertechnetate in the salivary glands of the rabbits were done. The rabbits were then sacrificed and their salivary glands were collected for histopathological examination. No significant changes were observed in serum SGOT, SGPT levels, salivary amylase and hemoglobin levels between the groups. ^{99m}Technetium pertechnetate uptake was seen in all the rabbits. Marked multifocallipomatosis and cellular atrophy was observed in the parotid glands of only^{131}I exposed rabbits whereas *O.sanctum* and amifostine pre treated rabbits exhibited cell architecture similar to the control group. As per the result above *O.sanctum* pre supplementation suggests radio-protective properties comparable to amifostine.

P051

Functional Annotation of Hypothetical proteins of *Neisseria gonorrhoea*: A Bioinformatics Approach for Identification of Newer Therapeutic Targets

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Neisseria gonorrhoeae has developed resist anceagainst various antibiotics making the cure difficult. This suggests the paucity of identification of novel drug targets and vaccine candidates to control the spread of disease. Functional annotation of Hypothetical Proteins (HPs) will help in identifying newer unexplored therapeutic candidates. Sequence of HPs of *Neisseria gonorrhoeae* was retrieved from ncbi database and various web-tools (CATH, CADD, systers, SVM prot etc.) were used for functional annotation. Protein-protein interaction was studied using SRING software. Essential and genes non homologues to genome of *Homo sapiens* were searched to identify potential therapeutic candidates.Three dimensional structure of two potential drug targets was modelled and extensive search against drug library was performed using PARDOCK.Thirty seven HPs out of 206 HPs were successfully annotated and characterised and grouped as enzymes, autotransporters and lipoproteins. Enzymes include glycosyltransferases, DNA modifying enzymes and metabolically important proteins. Protein-protein interaction studies supported the predicted function and presence of protein in a metabolic pathway. Four cytoplasmic HPs were found to be essential and predicted drugs were identified against two of them.These will be further checked using other docking tools and checked for cytotoxicity Furthermore, five extracellular HPs were found to be virulent suggesting their importance in pathogenesis using Virulentpred and checked as potential vaccine candidates by identifying B and T cell epitopes.The analysis of sequence-function relationships of HPs provides better understanding of the molecular machinery of the pathogen as well as suggested newer therapeutic targets.

P052

Serum Para-Oxonase Levels in Patients with Liver Disease: A Laboratory Based Study

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Para-Oxonase is an anti-oxidant enzyme, which is synthesized in the liver and circulates in the plasma, tightly bound to HDL.The physiologic role played by Para-Oxonase in the liver is unknown although preliminary observations suggest that this enzyme provides hepatic protection against oxidative stress. Aim was to estimate the Para-Oxonase activities in subjects with hepatic dysfunction and study the correlation of this enzyme with markers which are done routinely to assess the liver function. 50 Subjects diagnosed for liver disease with ALT values > 80U /L were included in the study. Control group consisted of 50 (corresponding age and sex matched) healthy subjects. Both, basal and salt stimulated Para-Oxonase activity was measured spectrophotometrically by the p-nitrophenol method. The liver function tests were performed on Roche P800 fully automated random access autoanalyser. The LFT profile included serum Total bilirubin, direct bilirubin, Total Protein, Albumin, Globulin, Alanine aminotransferase, Aspartate aminotransferaseand Alkaline phosphatase. A significant increase only in the salt stimulated PON in hepatic dysfunction was observed ($P<0.05$), without any changes in basal activity. The increase in ALT, AST, ALP, Total & Direct bilirubin was also statistically significant in the test group. Increased level of Para-Oxonase appears to be in parallel with the increase in ALT, AST & ALP & could be used to assess the degree of liver impairment.

P053

How to "Switch Off" Autoimmune Diseases- A Review

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Autoimmune diseases arise from an abnormal immune response of the body against substances and tissues normally present in the body (autoimmunity). It develops when our immune system which defends our body against disease, decides our healthy cells are foreign eventually attacking healthy body cells. Multiple sclerosis (MS) is an inflammatory autoimmune disease in which

the insulating covers of nerve cells in the brain and spinal cord are damaged. This damage disrupts the ability of parts of the nervous system to communicate, resulting in a wide range of signs and symptoms. Though the exact cause is not clear, the underlying mechanism is thought to be either destruction by the immune system or failure of the myelin-producing cells. Scientists have made a path breaking discovery in the fight against enervating autoimmune diseases such as MS by revealing how to stop cells attacking healthy body tissue. Rather than the body's immune system destroying its own tissue by mistake, researchers have discovered how cells convert from being aggressive to actually protecting against disease. This review article gives latest insight about the widespread use of antigen-specific immunotherapy as a treatment for many autoimmune disorders in the near future.

P054

Haematological Changes in *Plasmodium vivax* Infection

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Malaria is an epidemiological problem in tropical region. The World Health Organization estimated that over 300 million new cases of malaria arise per year, with ~ 2 – 3 million deaths resulting from contraction. Present study was aimed to determine specific changes in haematological parameters associated with the *Plasmodium vivax* infection.The study was carried out from 54 blood samples of malaria patients identified by the immunochromatographic *P. vivax* antigen test and peripheral blood smear examination in between August'2012 to June'2013. Haematological parameters were quantified by Sysmex XT 4000i auto analyzer, among which platelet were checked microscopically. *P. vivax* infection was most prevalent on August to September (46.3%) that declined to minimum during January to February. Malaria was widespread in female (64.8%) than in male (35.2%) patients. Infection rate was higher in adults, especially age group between 21 to 30 years (22.6%) and 61 to 70 years (17%). Anemia was manifested within 80% of male and 94.7% of female malaria patients. RBC count was observed below the reference level within both male (54.3%) and female (46.7%) patients. All female and 94.7% of male patients exhibited elevated erythrocyte sedimentation rate (ESR) during the course of infection. Thrombocytopenia was evident within 28.6% of malaria patients. Most of *Plasmodium vivax* infected cases in this study were characterized by anemia, reduced RBC count, elevated ESR and thrombocytopenia as common haematological changes. This may be helpful in prognosis of malaria and rapid commencement of the therapy.

P055

Various Gynaecological Disorders and Haematological Abnormalities in HIV Seropositive Women

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Gynaecological problems including menstrual disorders, vaginal infections, PID, STD, cervical dysplasias are common among HIV-positive women. Haematological abnormalities are a common complication of HIV infection involving all lineages of blood cells. HIV infects CD_4 T-lymphocytes, monocytes, and macrophages. Though a considerable number of studies are available in western literature on HIV and related gynaecological problems and prevalence of anemia in HIV women, very few studies are available from this part of the world. HIV-positive women attending the outpatient clinics at PGIMS Rohtak were evaluated to study these problems and to note the relation of above conditions with CD4 count and ART. A total of 200 HIV seropositive females in the age group 18 to 50 years attending the ART clinic were studied. Serological reactivity to HIV-1 and 2 was determined by enzyme immunoassay tests. After an informed consent all women were interviewed regarding their menstrual, medical and treatment history. Pelvic examination, cervicovaginal pap smears, papanicolauu staining was done. Blood samples were taken to determine the CD4 lymphocytes count using standard flow cytometric technique. Haematological Profile was studied in all the patients.The HIV seropositive women had a higher incidence of menstrual disorders (23.5%), vaginal infections (candidiasis-43.2%, bacterial vaginosis-47.7%, trichomoniasis-3%), cervical dysplasias which were worse with increasing immunosuppression (at lower CD4 levels: 19.4% and 7.3% respectively). 70% patients had anaemia especially in cases with increasing immunosuppression. Serum folate and serum ferritin levels were significantly lower, more so at lower CD4 levels. Serum iron levels were higher at lower CD4 levels. The screening of HIV seropositive women should be carried in a large number of patients.

P056

Genotypic Characterization of *Salmonella* Typhi 'Vi' Antigen Specific Bacteriophages for their Possible use in Clinical Practice

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Considering the high endemicity of typhoid in our country and the feasibility of phage therapy for this disease, we planned our study to explore the genomic structure of bacteriophages specific to 'Vi' positive *Salmonella* Typhi. Objective was to apply the different molecular tools to characterize *Salmonella* Typhi 'Vi' antigen specific bacteriophages genotypically. *Salmonella* Typhi 'Vi' antigen specific bacteriophages were isolated and propagated. Phage DNA was extracted and analysed by spectrophotometry, agarose gel electrophoresis and restriction enzyme digestion.In this study, we isolated two bacteriophages targeted against 'Vi' positive *Salmonella* Typhi. DNA of the phages was extracted for molecular characterization. On restriction analysis, both the phages were found to be of similar pattern, suggesting that they were genetically similar, although they had different plaque morphologies.The present study may be helpful in genotypic characterization of *Salmonella* Typhi 'Vi' antigen specific bacteriophages for their possible future prospect in clinical use.

P057

Potential Effects of Iron Status on Pulmonary Tuberculosis in Response to Directly Observed Treatment Short Course (DOTS)

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Among the many micronutrients, iron has a significant role in the pathogenesis of tuberculosis. Contribution of iron status to the underlying anaemia of chronic disease and its effects on the outcome is difficult to assess. Aim was to assess the potential effects of iron status on pulmonary tuberculosis in response to treatment. Non-Randomized controlled comparative prospective study was done,ninety newly diagnosed pulmonary tuberculosis cases were selected based on sputum smear positive & chest X-ray reports and were followed up till they completed the intensive phase of DOTS therapy. The patients were divided equally based on their baseline haemoglobin levels as anaemic (males: 9 – 12g/dL; females: 8-11g/dL) and non-anaemic (males: >12g/dl; females: >11g/dl). The iron status was assessed by estimating serum iron profile, ferritin and C- reactive protein (CRP) at baseline and after completion of intensive phase therapy. Serum iron, TIBC, transferrin saturation, significantly increased ($P< 0.001$), followed by significant decrease in S. ferritin and CRP ($P< 0.001$) in both the groups at the end of intensive phase therapy. 55.6% in the anaemic group and 51.1% in the non-anaemic group remained iron-deficient as indicated by the transferrin index (<1.0). 15 cases tested sputum smear positive (5 in anaemic group, 10 in non-anaemic group)at the end of follow up period. At the end of intensive phase therapy, improvement of BMI, iron status and CRP was observed in both the groups to a similar extent without any iron supplementation. However, more people in the non-anaemic group remained sputum smear positive at the end of DOTS therapy indicating that co-existence of anaemia was not a hindrance to the outcome.

P058

Biochemical and Hematological Alterations Due to Toxicity of Organophosphate Pesticide *Rogor* in Fish *Clarias Battrachus*

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In Agriculture, organophosphate pesticides are used to increase the yield of agricultural products and destruction of organism interfering with human food. The long term exposure of pesticides even at low levels may cause health hazards in human and other organism. Rogor is organophosphate pesticide with broad spectrum activity. The toxic mechanism of organophosphate compound is based on the irreversible inhibition of acetyl cholinesterase due to phosphorylation of the active site of the enzyme. The main objective of the study was to evaluate biochemical parameters of serum cholesterol, SGOT and hematological parameters of hemoglobin and total leucocyte count in fish clarias Battrachus exposed under organophosphate pesticide Rogor. The study was performed in fish Clarias Battrachus exposed 24 to 96 hours to four different concentrations of Rogor Pesticide. Serum cholesterol, SGOT levels were estimated as biochemical biomarkers while Hemoglobin, TLC was determined as hematological biomarkers. A significant rise in serum cholesterol and SGOT as biochemical markers and Leukocytosis, hemoglobenemia as hematological biomarkers were observed in Rogor pesticide exposed on clarias Battrachus. From the present study it can be calculated that hypercholesterolemia and elevated SGOT may lead to disorders like Myocardial Infarction, Heart Attack, Atherosclerosis, CAD etc., while leukocytosis and hemoglobinemia may lead so many health hazardous disorders. Therefore it is advised to pesticide workers

to take all precautions regarding protection from pesticide exposure and proper use of prophylactic supplementation for healthy life.

P059

Serum Vitamin C Levels in Non-Alcoholic Chronic Smokers

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Vitamin C, also known as ascorbic acid, is a water-soluble vitamin and is essential for normal functioning of the body. When comparing smokers with non-smokers, evidence consistently indicates that current smokers have lower blood levels of vitamin C. Additionally, studies have shown decreasing vitamin C levels as the number of cigarettes smoked per day increases. To estimate serum vitamin C level in non-alcoholic chronic smokers and compare it with non-smokers.The study was conducted in the Department of Biochemistry, on the staff members and students of Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Ambala. Total number of 60 male subjects between the age of 18 to 60 years were selected and divided into two groups. Group 1: 30 subjects; healthy non-alcoholic chronic smokers and Group 2: 30 subjects, healthy non-alcoholic non-smokers. 5 ml of fasting venous blood was collected from antecubital vein under aseptic conditions from each subject into plain vials. Serum was separated and vitamin C was estimated by colorimetry. Significantly lower serum vitamin C levels were observed in smokers (0.24 ± 0.18 mg/dl) as compared to non smokers (1.38 ± 0.51 mg/dl, P<0.001). The age, body weight, height and Body Mass Index (BMI) did not affect the level of vitamin C. Smokers have a significantly lower level of vitamin C as compared to non-smokers and may need supplementation.

P060

Correlation between Periodontal Clinical Parameters and Biochemical Systemic Oxidative Stress Markers in Patients with Type 2 Diabetes Mellitus and Chronic Periodontitis

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Chronic periodontitis and diabetes have mutual influence and a bidirectional relationship via oxidative stress. In sync with an emerging interest among researchers to find association between periodontitis: an oral disease and diabetes: a systemic disease, the present study assesses correlation if any among clinical periodontal parameters and some systemic oxidative stress markers in patients with type 2 diabetes mellitus and chronic periodontitis. In this cross-sectional study a total of 60 patients with generalized chronic periodontitis (clinical attachment level =3; American Academy of Periodontology 1999 criteria) were enrolled and divided into two groups; Group I (CP) - non diabetics with chronic periodontitis (fasting glucose=6.1 mmol/L) (n=30) and Group II (CPDM) - type 2 diabetics with chronic periodontitis (fasting glucose=7.0 mmol/L; WHO 2006 criteria) (n=30). Periodontal status was evaluated using gingival index (GI), plaque index (PI), probing depth (PD) and clinical attachment loss (CAL).The biochemical parameters estimated were fasting glucose, total antioxidant capacity (TAOC), RBC- superoxide dismutase (RBC-SOD), glutathione peroxidase (GPx), ascorbic acid and Malondialdehyde (MDA). Among the biochemical parameters, the Pearson correlation test showed significant (P< 0.05) negative correlation of TAOC, ascorbic acid and positive correlation of RBC-SOD, fasting glucose to clinical parameters. Under the study conditions, our results point to a possible correlation between oral clinical parameters and systemic biochemical markers. The clinical damage to periodontium is associated with alterations in systemic oxidative stress markers among patients with type 2 diabetes and chronic periodontitis. Thus, emphasizing the need of professional care to the oral condition in patients with diabetes.

P061

Modulatory Effect of Whey Preparation on Brain Thiols of Rats Exposed to Etoposide

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Chemotherapy is known to adversely affect the brain tissue and alter the enzyme system. The mechanism(s) associated with chemotherapy induced altered enzyme system remains obscure. Few studies point to the total thiols which is an important molecular target for this toxicant. The present study investigates the effect of Whey preparation on GSH, GST, Total Thiol level in rat brain exposed to etoposide. All rats were randomly divided into three groups. The first group served as the normal control. The second group was treated with etoposide (60 mg/kg body weight) intraperitoneally. And the third group was treated with etoposide along with Whey preparation (100 mg/kg body weight) for 72 hours. Animals were sacrificed by decapitation after 72 hours. Tissue samples were collected and homogenate was done with respective buffers and were stored at -20^{o}C until use. Treatment efficacy was assessed by changes in the brain biochemical parameters such as GSH, GST and total thiols in control, etoposide, and Whey preparation treated group.There was a significant decrease in GSH and total thiols level as well as GST activity when compared to etoposide group. Whey preparation intervention group showed a significant increase in GSH & Total thiols level when compared with etoposide treated group. However GST activity showed a tendency to revert back towards normal. These data suggests that chemotherapy affects total thiols which are responsible for functional thioltransferase system in rat brain.

P062

Oxidative Stress and Antioxidant Parameters in Sickle Cell Anemia Patients of Central India

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Sickle cell disease refers to a genetic disorder characterized by a haemoglobin variant called HbS. The prevalence of sickle haemoglobin from various parts of Madhya Pradesh and Chhattisgarh varied from 15 to 30 percent. A high production rate of reactive oxygen species in Sickle cell disease caused by several factors such as chronic inflammation, intravascular hemolysis, ischemia reperfusion injury and decreased level of antioxidants. Aim is to know the status of antioxidant and oxidative stress in the sickle cell patients.The study was carried out in Chhattisgarh Institute of Medical Sciences, Bilaspur (C.G.). The study was conducted on 90 human subjects. out of which 30 were suffering from Sickle cell disease, 30 were Sickle Cell Trait and 30 were taken as control group (Healthy age matched, having no blood disorder). The written consent was also obtained before starting the study. All the Biochemical tests were carried out in all subjects by using standard methods and statistically analyzed**.** Antioxidant enzyme CAT and GPx were significantly decreased except SOD ($P<0.001$). MDA use as oxidative marker and found significantly increased ($P<0.001$) in the sickle cell anemia groups as compared to sickle cell trait and healthy subjects. Thus, in our study, we observed enhanced oxidative stress in both homozygous and heterozygous SCA patients as compared controls which can increase the severity of disease. Endogenous mechanism appeared to be failure to mop up reactive oxygen species.

P063

Effect of Different Physical Factors on Oxidation of Edible Oils Commonly used in India

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Since past few decades modern epidemics like CVD, obesity, metabolic syndrome and cancers are at an alarming increase. Reasons being variable from changed lifestyle, altered food habits and environmental changes. Objective was to combat with such disorders various steps have been undertaken one of which is replacement with PUFA rich edible oils in our diets. But lipids containing PUFA are highly susceptible to oxidation,which may take place during processing, storage and exposure to temperature during different modes of cooking. Present study was undertaken to see the effect of different physical factors on oxidation of edible oils. Oils were exposed to different storage condition and temperature and seen for their OD, Iodine number, smoke point, primary and secondary oxidation products and were correlated to the oxidation products generated. Optical density was found to be decreasing under the effect of sunlight and temperature ($P<0.001$). PV values were increasing under the effect of sunlight and temperature where as AV and TBARS were increasing when primary oxidation products were decreasing. PUFA in the edible oils undergoes oxidation, which is maximum under the effect of sunlight and high temperatures. Study throws light on the method of cooking and storage conditions most suitable with minimum oxidation product formation.

P064

Neuroprotective Potential of *Bacopa monnieri* Plant Extract in MPTP Induced Mice Model of Parkinson's Disease

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Parkinson's disease (PD) results primarily from the death of dopaminergic neurons in substantia nigra. Some studies are being conducted to find a suitable and effective cure for PD, with an emphasis on the use of herbal plants (*Bacopa monnieri*), but the phenomenon of neurogenesis is not well-known.In this study, we tried to explore the neuroprotective & neurogenic effect of *Bacopa monnieri*plant extract (BME) in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)induced mice model of Parkinson's disease (PD). Study comprised of twenty four Swiss albino mice (35-45 gms), which was as follows: Control, BME, MPTP and MPTP+ BME (6 mice in each). Experimental mice were given 40mg/kg body weight BME treatment orally for one month with prior use of 15mg/kg bw of MPTP for two weeks. After that, behavioral study was performed and assessment of neuroprotective effect was studied via biochemical analysis, immunohistochemical parameter which included functional viability of dopaminergic neurons in substania nigra by Tyrosine hydroxylase (TH) using monoclonal antibody against TH & m-RNA expression of neurogenic genes/neuronal transcription factors in the hippocampus and substantia nigra region of brain. A significant recovery in Spontaneous loco-motor activity, lipid peroxidaton and conjugated dienes levels was evident in MPTP+BME group as compared to MPTP treated animals. In MPTP treated mice, number of surviving TH positive neurons were significantly less as compared to others groups. MPTP+BME group exhibited a significant increase in TH-immunoreactive neurons when compared to control group ($P< 0.001$). In m-RNA expression analysis a significant change was found in MPTP + BME when compared to control. Our results revealed the role of BME in reducing the oxidative stress prevents dopaminergic neurodegenration & restores the cell loss thereby proving its neuroprotective potential. Results support further investigations on this plant, and its active constituent compounds, as possible therapeutic intervention against Parkinson's disease.

P065

Interrelation of Glutathione-S-Transferase and Reduced Glutathione in Diabetic Patients with and without Nephropathy

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Hyperglycemia in diabetes mellitus (DM) is associated with increased production of reactive oxygen species (ROS) which rapidly interact with proteins, lipids, and carbohydrates and may cause renal damage. Glutathione-S-Transferase (GST) is primarily involved in the neutralization of harmful exogenous or endogenous compounds by enzymatic conjugation with the scavenger peptide glutathione (GSH). Hence, it is involved in combating oxidative stress.The present study was aimed to assess the activity of GST, reduced glutathione (GSH) levels and their correlation in patients with Type 2 diabetes mellitus with and without nephropathy.This study comprised of 300 participants divided into three groups of 100 each: Type 2 diabetic patients (T2DM) without nephropathy, Type 2 diabetic patients with nephropathy (T2DM–CKD) and healthy controls (HC). Plasma GST activity and blood GSH levels were estimated spectrophotometrically. Highest GST activity was observed in DM (8.50±0.55) which was significantly higher as compared to DM-CKD (7.39±0.50; $P<0.05$) and HC (6.36±0.69; $P<0.05$). GSH levels were lowest in DM-CKD (0.9±0.17) as compared to DM (1.79±0.17; $P<0.05$) and HC (3.21±0.24; $P<0.05$). GST and GSH showed a significant negative correlation in DM. Though, the trend was similar in DM-CKD group, however it was not statistically significant. These results suggest that increased activity of GST is associated with decreased GSH levels in diabetic patients, leading to disturbed redox milieu which collectively conspire to progressively increase the risk of renal damage in diabetes mellitus.

P066

Insight to Preeclampsia- An Association of Platelet Factor-4 and Nitric oxide (NO)

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Platelets play a crucial role in the pathophysiologic mechanisms of preeclampsia. Platelet-derived Nitric Oxide (NO) is known to inhibit platelet activation and prevent thrombus formation.

Impaired platelet NO production increased P-selectin expression on the thrombocyte surface resulting in enhanced platelet adhesion to monocytes and elevated expression of tissue factor, an initiator of coagulation. Our purpose was to investigate the correlation between Platelet nitric oxide and Platelet Factor-4. Total of 500 subjects were taken (200-control subjects, including healthy pregnant females & 300 study subjects diagnosed with preeclampsia) from the wards of SSKH & LHMC, with informed consent from subjects, after approval by institutional ethical committee. The blood was taken and according to the protocol the Platelet rich plasma was taken for the detection of PF4 & NO by ELISA & spectro-photometrically respectively. In the present study significantly high levels of PF4 ($P<0.001$) were found in study subjects as compared to control and opposite trends of NO ($P<0.001$) were seen. In the study, Positive correlation between NO & PF4 was found ($P<0.01$).High levels of platelet proteins emphasize the active role of the platelets in the alterations of hemostasis in cases of preeclampsia.

P067

Effect of Low Dose Ionizing Radiation on Age Related Diseases

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Humans are constantly exposed to low doses of ionizing radiation (IR) deriving from background radiation (cosmicrays, radon), outer space sources, occupational and medical purposes. Absorption of ionizing radiation doses over a prolonged period of time can result in oxidative damage and age related diseases. Aim was to discuss the risk of age related diseases after a prolonged exposure to low doses of ionizing radiation through environmental, occupational and medical diagnostics. Recent studies have examined the risk of age related diseases in humans exposed to various sources of radiation, such as medical treatments, radio-surgery, medical imaging and space flights. These studies clearly indicate the relationship of low doses ionizing radiation and formation of free radical species, causing oxidative cell damage and subsequent age related complications through alterations in biochemical pathways because they are sensitive to theactions of oxidants. As per the results of various research studies we have concluded that there is a strong association between prolonged low doses ionizing radiations and age related diseases.

P068

Biochemical Studies on Circulating Plasma Lipid Peroxides, Status of Oxidative Stress Markers, Antioxidant and Enzymes in Alcoholic Liver Diseases

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Alcoholic liver disease is often progressive and is considered to be a major cause of morbidity and mortality. The Antioxidant status of pro-oxidant in alcoholic liver disease among the chronic alcoholics is still not clear. Studies have shown that ethanol consumption may result in increased oxidative stress with increased formation of lipid peroxides and free radicals. The present study was conducted in Department of Biochemistry, H.N.B Base Hospital, Srinagar, Garhwal, Uttarakhand. 50 Alcoholic Liver Disease patients were subjected to detailed clinical examination and laboratory investigations and the results were compared with 30 controls. Blood samples were collected for oxidative stress parameters. It was observed that there was a significant increase in activities of SOD, GPX and a significant decrease in erythrocyte ascorbic acid, plasma vitamin E levels and catalase activity in patients with ALD when compared to controls. Results of our study depict higher oxygen free radical production, evidenced by increased levels of MDA and decreased levels of GSH, ascorbic acid, vitamin E and Catalase activity, supporting the evidence of oxidative stress in Alcoholic patients. Decreased concentrations of antioxidant vitamins support the hypothesis that Alcohol is an important causative factor in pathogenesis of lipid peroxidation. These data reveal that antioxidant defense mechanisms might be impaired in patients with Alcoholic Liver Diseases. These findings also provide a theoretical basis for development of novel therapeutic strategies, such as antioxidant supplementation.

P069

Moderate Alcohol Consumption and Oxidative Stress

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Oxidative stress is major cause for number of diseases. Moderate alcohol consumption has various favourable metabolic changes. The mechanisms for these metabolic changes are largely unknown. Thus, this study was intended to evaluate oxidative stress

and enzymatic antioxidants in subjects consuming moderate alcohol.To estimate lipid perxoidation (LPO) and activity of antioxidant enzymes in moderate alcohol consumption. The study included two groups each with 50 healthy male. Group I consuming 90 mL whiskey/day, Group II consuming 180 mL whiskey/day and 50 healthy non alcoholic males were control. Serum was used to estimate Malondialdehyde (MDA) by Kei Satoh method and Erythrocyte activity of Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Catalase were measured by Randox, Ransel and Aebi H method respectively. There was no significant difference in levels of LPO between controls and group I (MDA3.85±1.23 / 4.10±1.8 nmol/ml P value >0.005) and significantly increased LPO in group II than controls (MDA 5.62±1.85/3.85±1.23 nmol/ml P value <0.0001). The enzymatic antioxidant activity was significantly increased in group I in comparison with controls (SOD 184.6±15.6 / 140±18.4 U/mL, GPX 8842 ± 2584 / 6864±1840 U/ml, Catalase 30.80±4.6 / 26.84±3.8, P value <0.0001). The enzymatic antioxidant activity was found to be significantly decreased in group II in comparison with controls (SOD 125.6±16.8/ 140±18.4 U/mL, GPX 4486±1645 / 6864±1840 U/ml, Catalase 22.6±4.1 / 26.84±3.8, P value <0.0001). In group II oxidative stress might increase the consumption of antioxidant enzymes. Study concluded that moderate alcohol consumption 90 ml/day increases the activity of enzymatic antioxidants.

P070

Oxidative Stress in Periodontitis in Haryana

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Periodontitis is a chronic inflammatory disease associated with gram-negative bacteria characterized by connective tissue and alveolar bone destruction. Though many studies have revealed the alterations in the salivary NO and MDA concentrations, desperately only few studies have reported simultaneous estimation of the serum NO and MDA levels. Therefore present study was planned to estimate the level of NO and MDA in patients of periodontitis in population of Haryana. A total of 50 subjects were included in the study. Out of these, 25 were healthy controls and 25 were periodontitis patients. The NO level (measured as nitrite-plus-nitrate (NO(x)) concentration) was estimated by Griess reagent method. Serum MDA level was estimated by thiobarbituric acid (TBA) reaction. Patients in periodontitis group showed a significant increase in serum MDA levels and NO levels when compared to controls (P< 0.05 and < 0.001 respectively). As per Pearson's correlation coefficient, serum NO levels were found to be positively correlated with serum MDA levels in periodontitis patients but the correlation was not significant statistically. Patients with periodontitis show higher systemic oxidative stress and inflammation as compared to healthy controls. NO and MDA can be considered as a biological marker, the presence of which in serum can partially determine the extent of periodontitis. Finally the use of NOS inhibitors and antioxidants can prove beneficial in limiting the progress of disease.

P071

Evaluation of Protein Carbonyl Content and Total Antioxidant Status in Pre and Post-Delivery of Women with Pre-eclampsia within 48 hours

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Preeclampsia is a metabolic syndrome and a major cause of maternal, fetal and neonatal morbidity and mortality that amounts to 10% of the pregnancy complications. Objective was to determine protein carbonyl and total antioxidant status in preeclampsia and normal pregnant women during pre and post labour within 48 hours. A prospective case control study preeclampsia (n=30) and normal pregnant women (n=30) during pre and post labour within 48 hours. 3 ml of blood samples were collected during pre and post labour of normal pregnant and pre-eclampsia. Protein Carbonyl Content and Total Antioxidant Status (TAS) were measured using spectrophotometric method.The Mean ± SD values of Protein Carbonyls 168.9±70.5 nmol/L, TAS 537 ± 451 mmol/L in normal pregnant during pre-delivery and 169±67.2 nmol/L, 634.3 ± 241.2 mmol/L post-delivery presented. Similarly Mean ± SD values of Protein Carbonyls 159±123.2 nmol/L, TAS 506.7 ± 287.6 mmol/L in preeclampsia pre delivery and 98.8±36.8, 680±362.3 post-delivery respectively. Decreased total antioxidant status observed in pre-eclampsia in comparison to control group in pre delivery. However protein carbonyl is unaltered in control group but decreased in pre-eclampsia after delivery. A well-known aspect in pre-eclampsia is increased concentrations of oxidative stress and decreased TAS. However the trend of increased TAS and declined oxidative stress during post labour of normal pregnant and pre-eclampsia which plays a significant role in pathophysiology of preeclampsia.

P072

To Establish the Reference Range of Free Thyroid Hormones

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Thyroid disorder is common worldwide. In India, there is significant burden of thyroid disease. According to the projection from various studies, it has been estimated that 42 million people in India suffer from thyroid disease. Usually, total serum T3, T4 and TSH are used in biochemical analysis. Gradually, the trend is shifting from total serum T3, T4 to free T3 (FT3), free T4 (FT4) which are biologically more active. However, the reported reference range varies so that every laboratory has to establish its own reference range. Aim was to establish the reference range of serum FT3 and FT4 in our hospital laboratory and compare it with the values reported by other standard laboratories.The study was conducted in the Department of Biochemistry, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Ambala, Haryana. 32 apparently healthy euthyroid subjects were selected and divided into 2 groups. Group 1: 7 male subjects; Group 2: 25 female subjects. 5 ml of fasting venous blood was collected from antecubital vein under aseptic conditions from each subject into plain vials. Serum was separated and FT3, FT4, TSH were estimated by ELISA. In females, serum FT3 levels were 2.55±1.16 pg/ml, FT4 levels were 1.23±0.37 ng/dl and TSH levels were 2.40±1.56 μIU/ml, whereas the corresponding values in males were 2.93±1.11 pg/ml, 1.26±0.39 ng/dl and 1.92±2.86μIU/ml respectively. Overall ranges were 2.64±1.17 pg/ml, 1.23±0.42ng/dl and 2.75±2.87μIU/ml respectively which are comparable (P>0.05) to those reported by other laboratories, e.g. Lal Path Lab (FT3 = 1.4-4.2 pg/ml, FT4= 0.8-2.0 ng/dl, TSH=0.28-6.82 μIU/ml), Thyrocare (FT3=1.7-4.2 pg/ml, FT4=0.70-1.80 ng/dl, TSH= 0.30-5.5 μIU/ml) and SRL (FT3=2.50-3.90 pg/ml, FT4=0.66-1.20 ng/dl, TSH=0.34-5.60 μIU/ml). Our laboratory levels of free thyroid hormones were comparable with other reference range of different laboratories and thus suitable to be used as reference during analysis of patient samples.

P073

Association of Osteocalcin with Different Parameters of Diabetes Mellitus

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As it has been reported that the skeletal system also plays a role in the regulation of energy and glucose metabolism and it has been shown in experimental animals that recombinant osteocalcin administration increases insulin sensitivity and adiponectin levels. Study the association of osteocalcin with various parameters of diabetes mellitus. 38 young healthy non-diabetics off springs of diabetic parents in age group between 18 to 30 years were enrolled. Socio-demographic data were entered in semi structured proforma. Weight and height were recorded. BMI and Waist hip ratio was calculated. Fasting blood sample was collected for blood glucose, insulin and lipid profile. Blood glucose and lipid profile was estimated by fully auto-analyzer A25. Insulin was estimated by ELISA method. Insulin resistance was calculated using HOMA-IR. Serum osteocalcin was measured by ELISA method.In our study we found mean value of osteocalcin (14.1±1.2). We also found that serum osteocalcin has significant (r=-0.440 at P<0.01) negative association with BMI, it was also negatively associated with W/H, FBS and insulin resistance but not clinically significant. In our study serum osteocalcin was positively associated with serum cholesterol and insulin but it was not significant. In our study we were able to derive mean value of serum osteocalcin in young healthy non-diabetic offspring of diabetic parent. We also calculated the value of different parameters of diabetes mellitus in this young adults**.** We found significant positive correlation of serum osteocalcin with BMI.

P074

Salivary Biomarkers: Potential Diagnostic and Prognostic Tool in Type II Diabetes Mellitus

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Management of type II Diabetes Mellitus in evidence based medicine is greatly dependent on patient friendly diagnostic modalities. Saliva, which contains various serum constituents, can serve as a mirror to the systemic disorders especially Diabetes Mellitus. This widely abundant fluid with its biochemical markers can be a promising tool for diagnosis and monitoring. The routinely used blood parameters in type II diabetes have been scarcely studied in saliva. The purpose of the study was to understand and establish the role of saliva with its biochemical markers as a diagnostic and prognostic tool in type II Diabetes Mellitus. The study aimed to correlate the routine investigative parameters in blood with their levels in saliva in the subjects. A case – control study was done including 40 type II diabetic patients (age 35-65 yrs) as cases and 40 age matched non diabetics as controls. The study excluded the subjects with any known hepatic & or renal disorders. Both the groups were investigated for blood and saliva levels of glucose, total protein, sodium, potassium and calcium. In addition, blood samples were also checked for HbA1C, SGPT & Creatinine and Saliva samples were checked for pH & buffering capacity. The results were found to be significant. The levels of HbA1C were significantly correlating with salivary glucose levels and the

physical properties of saliva. These non- invasive salivary biomarkers shall facilitate the treatment, prevention of progression and complication of diabetes.

P075

Subclinical Hypothyroidism and it's Association with Anti TPO in Western U.P.

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Thyroid disorder is considered to be one of the most common endocrine disorders worldwide. Hypothyroidism constitutes a major chunk of this common non communicable disease. The prevalence of hypothyroid has been estimated to be 4-5% in developed countries with speculated prevalence of 4-15%. of Subclinical Hypothyroidism in these countries. In India, the estimated prevalence of thyroid disease is 42 million seen in various studies.The present study was done to find an association of Subclinical Hypothyroidism with Anti TPO antibodies.This retrospective study was carried out in 276 non pregnant women of = 18 yrs attending a teaching Hospital in Western UP. The study subjects were analysed in two groups; Group A: 120 Subjects who has under gone thyroid profile test which includes TSH, T3, T4 And Group B: 156 Subjects who has under gone Thyroid Profile Test which includes TSH, fT3, fT4. Forty subjects which are diagnosed as Subclinical Hypothyroidism were subjected to Anti TPO antibodies analysis. Hormonal Analysis was done on MINIVIDAS using Enzyme linked Fluorescent assay Techniques and Anti TPO antibodies were analysed by ELISA (Euroimmune, Germany). Of the Group A, 89 (74.2%) Subjects were Euthyroid; 20 (16.7%) Subjects were subclinical Hypothyroid. In the Group B: 119 (76.3%) Subjects were Euthyroid with and 21 (13.5%) subjects were subclinical Hypothyroid. subsequent Anti TPO analysis of 40 subjects shows 15 (37.5%) had Anti TPO positivity and 25 (62.5%) were Anti TPO negative. With higher prevalence in Elderly Subjects P=0.000 (t=10.68, df=13, P<0.01). Subclinical hypothyroidism patients have significant prevalence of anti TPO antibodies. So this test must be considered for evaluating the clinical abnormalities including infertility

P076

Cortisol Levels in Patients with Mild Cognitive Impairment and Alzheimer's Disease

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Elevation of serum cortisol is reported in Alzheimer's disease (AD). AD and Mild Cognitive Impairments (MCI) are stressful situation, hence are prone to hypercortisolemia. Serum cortisol has been proposed as a marker of cognitive decline.Aim is to study frequency of cortisol level in AD/MCI.We studied 28 Patients (M=24:F=4), presenting with subjective memory complaints.There were a total of 15 patients with MCI and 13 with AD (mean age 73.39 ±7.6 years) and duration of illness was 3.4±3 years. Mean Mini Mental State Examination (MMSE) Score was 21.7±7.4. Morning 8 AM blood sample for cortisol was taken usingChemiluminescence Based Competitive Assay(Reference Range 10-20 microgram/dl). A total of 4 (14%) had a high morning serum cortisol. Only one case out of 4 was MCI, rest had AD (Mean MMSE=15). Hypercortisolemia has been seen in AD/MCI. Dementia severity is associated with a rise in serum cortisol. In our cases too, higher serum cortisol was found in patients with Dementias, compared to MCI. Higher serum cortisol is associated with rapid disease progression. Four patients with AD/MCI had higher range morning cortisol levels.

P077

Thyroid Dysfunction in Post Radiotherapy follow-up Patients of Head and Neck Cancer

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The reported incidence of hypothyroidism following surgery and/or radiationtherapy for head and neck cancer varies widely. Most patients undergo thyroid lobectomyduring laryngectomy. Standard radiation treatment portals often include the thyroid gland.The insidious development of hypothyroidism may be misdiagnosed. This study examinesthe incidence of thyroid dysfunction in the setting of head and neck cancer therapy. To analyze Serum TSH, Total T3 and Total T4 level and evaluate risk of in post radiotherapy follow-up patients of head and neck cancer.This case-control study was conducted on 25 known cases of head and neck cancer that had undergone radiotherapy at least 6 months back and were on follow-up at S.S.G. Hospital, Vadodara (Group I). 25 normal age & sex matched healthy individual were

taken as control group (Group II). In all these subjects, Serum TSH, Total T3 and Total T4 were measured on microplate ELISA Reader by ELISA technique. Significantly higher levels of S.TSH were found in post radiotherapy follow-up patients of head and neck cancer. Hypothyroidism is a common late effect after external radiotherapy for head and neck cancers. Routine testing for possible thyroid hypofunction should be included in the follow-up procedures. Prompt recognition and intervention may prevent or reverse adverse physiological outcomes.

P078

Role of Levothyroxine on Maternal and Fetal Outcome

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Pregnancy is associated with hypothyroidism**.** Objective was to find out the role of levothyroxine on maternal and fetal outcome in pregnant women.The present study was carried out in 200 pregnant women and thyroid function tests namely, TSH, FT_3, FT_4 were done. The patients were followed till delivery for perinatal outcome. Maternal and cord blood for TSH were collected at the time of delivery. Mean TSH levels at first trimester in Group 2 (case) were significantly higher than those in Group 1(control) (P = 0.04). The patients with raised TSH levels were given treatment with levothyroxine as per their TSH levels. The mean TSH levels at the time of delivery in group 2 patients were significantly lower as compared to the mean TSH levels in the first trimester and they were comparable to euthyroid patients (P-value=0.32). Cord blood TSH values were 4.47±2.1 mIU/L and 6.88±2.3 mIU/L in group 1 and group 2 respectively, P<0.01. The correlation between maternal TSH at first trimester and cord blood TSH was positive (r-value =+0.227, P<0.01). Negative correlation was observed between the TSH values in first trimester and average birth weight of neonates (r-value=-0.038, P=0.05). Negative correlation was observed between cord blood TSH and birth weight of babies (r value = -0.15, P<0.05). Subclinical hypothyroidism was associated with increased incidence of low birth weight babies and biochemical parameters and perinatal outcomes were better after thyroxine replacement therapy.

P079

Assessment of Insulin Resistance and HbA1c in Women with Polycystic Ovarian Syndrome

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Polycystic ovarian syndrome (PCOS), the most common cause of infertility, is a disorder characterized by chronic anovulation, hyperandrogenism, hyperinsulinemia, and often presence of obesity. One of the most common markers of chronic glycemia is hemoglobin Alc (HbA1c). The present study was designed to assess insulin resistance and glycated hemoglobin (HbA1c) in women with PCOS. A comparative study including 30 women diagnosed as PCOS and 30 age and BMI matched healthy women with normal menstrual cycle as controls was conducted. Height, weight, body mass index (BMI), fasting blood sugar (FBS), fasting serum insulin, glycated hemoglobin (HbA1c) and insulin resistance were determined. Insulin resistance was calculated by homeostasis model assessment (HOMA). A significant increase in fasting serum insulin (P<0.001) was found in PCOS subjects in comparison with controls. Similarly, a significant increase HOMA-IR was observed in PCOS subjects compared to controls (P<0.001). Mean BMI, FBS and HbA1c were found elevated in the PCOS women compared to controls but differences between the two groups were not statistically significant. Interpretation of data was done using SPSS version 13.The current study provides further evidence that significantly higher fasting insulin and HOMA in PCOS women indicates presence of IR and it may have a potential role in the prediction of dysglycemic disease in PCOS women. Combined HbA1c, BMI and serum insulin level provides a significant model with potential use to evaluate metabolic/vascular disease in PCOS women.

P080

Assessment of Serum Ferritin Levels in Patients with Hyperthyroidism

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Previous studies show serum ferritin measurement as a marker of thyroid hormone action on peripheral tissues. Also, there are several studies supporting the role of thyroid hormones in erythropoiesis and effect of thyroid status on iron metabolism of a person. This study was aimed to investigate an association between serum ferritin levels and hyperthyroidism.This was a cross-sectional study done in the department of Biochemistry, Pt. B.D. Sharma,

PGIMS, Rohtak. Thirty patients diagnosed with hyperthyroidism and thirty age and sex matched healthy controls were enrolled in the study after taking informed consent. Levels of ferritin, free T3 and free T4 in serum was analysed by competitive immunoassay using direct chemiluminescent technology on ADVIA Centaur CP Immunoassay and Thyroid stimulating hormone (TSH) using immune radiometric assay in both groups. Serum ferritin levels of hyperthyroid patients were higher than those of healthy controls ($P<0.05$). Free T3 and free T4 levels in hyperthyroid patients were higher than those of controls ($P<0.05$), but TSH levels were lower than only those of healthy control. Hyperthyroidism is associated with high serum ferritin levels. The estimation of serum ferritin may help in understanding pathophysiology of thyroid disorders and also diagnosis and monitoring of hyperthyroid patients.

P081

Effect of Thyroid Hormone on Glycated Haemoglobin (HbA_1C) Levels

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HbA_1C is widely used for assessment of glycemic control.In addition to blood glucose levels, HbA_1C is also affected by erythropoiesis. Alteration in HbA1C level in hypothyroidism may not be solely due to alteration in blood glucose. So, this study was done to determine the effect of thyroid hormone on HbA_1C levels. Objective was to determine HbA_1C levels in newly diagnosed non-diabetic hypothyroid cases and to compare them with HbA_1C levels in euthyroid controls and determine the effect of thyroid hormone on HbA_1C levels.This case-control study was conducted on 30 newly diagnosed cases of hypothyroidism at S. S. G. Hospital, Vadodara (Group I). 30 normal age & sex matched healthy individuals were taken as controls (Group II). In all these subjects, fasting blood samples were obtained for analysis of glucose, HbA_1C, TSH, total T3 and total T4. Serum thyroid hormones were measured on micro plate ELISA Reader. Glucose and HbA_1C were measured on fully automated biochemistry analyzer cobas c-311. HbA_1C levels were significantly higher in patients with hypothyroidism as compared to controls. We conclude that in hypothyroidism, HbA_1C level is increased even with normal blood glucose. Hence, the effects of thyroid hormone on HbA_1C must be considered while interpreting the results of this parameter in patients with thyroid disorders.

P082

Study of 25-OH Vitamin D and Insulin Resistance in Obese Adolescents

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Vitamin D deficiency in obese adolescent is strongly associated with increased risk for diabetes, hyper tension and metabolic syndrome. Vitamin D insufficiency is a risk factor for developing impaired glucose in childhood obesity and is associated with insulin resistance in obese adolescents. In our study, we examined the relationship between vitamin-D and insulin resistance in obese adolescents. Objective was to study the relationship between serum 25-OH-D levels and insulin resistance in obese and non obese adolescents.The study group included 50 obese adolescent aged (17-19 years) and compared with 50 age and gender matched controls. 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 level in obese adolescents is 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.

P083

Status of Anti-TPO Antibody and Thyroid Hormone in Idiopathic Chronic Urticaria

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Idiopathic chronic urticaria may be associated with other auto-immune diseases like autoimmune thyroiditis. Aim was to study the status of thyroid hormone and anti –TPO ab in patients of idiopathic chronic urticaria.The observational study was conducted from month of January to June 2014 in PGIMS Rohtak. The blood samples were collected from thirty diagnosed cases of chronic urticaria (group-a) and thirty age and sex matched control (group-b)Anti-TPO antibody (anti-TPO ab) was detected by chemiluminiscence enzyme immunological method, free T4 and free T3 by luminescence enzyme immunoassay. TSH was estimated

by RIA. Autoimmune thyroiditis was considered on basis of anti-TPO >220 mUI/ml.The mean age of patients was 31 years. Male feamle ratio is 1:2.9. Mean value of Anti-TPO was 237.93 mUI/ml in group-a and 44.85 mUI/ml in group-b. The values of anti-TPO ab was significantly higher in group-a than group-b (P<0.01). Mean ± SD values of TSH in group-ais 7.56±2.901 and 1.72±0.147 respectively. The values of TSH was significantly high in group-a than group-b (P< 0.01). The difference of FT3 and FT4 between the groups were not statistically significant. This study shows a statistically significant association between hypothyroidism and chronic urticaria. Full thyroid profile (serum thyroid autoantibodies, serum TSH, FT3 and FT4) is highly recommendedin patients with diagnosis of chronic urticaria.

P084

Reproductive Hormones Imbalance in HIV Seropositive Males

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This study tested the clinical utility and relevance of analyzing reproductive hormones (testosterone, estrogen, progesterone) in HIV seropositive males and correlation between hormone levels and their CD4+ cell counts. Fifty three HIV seropositive males attending ART centre of PGIMS, Rohtak were included in this study. On the basis of CD4+ cell counts three groups were divided : Group A-CD4 cell counts < 200/mm3, Group B-CD4 cell counts 200-350/mm3, Group C-CD4 cell counts>350/mm3. Serum testosterone, estrogen and progesterone levels were measured and compared with fifty age and sex matched controls. Mean age was 32 ± 7 years in males with a range between 17-45 years. Mean testosterone level was significantly decreased at low CD4 counts in male cases than controls. Significant decrease in testosterone level in males was seen in comparison between Group A vs control (233±100.89 Vs 534.97±152.54), Group B (296.45±165.04) vs Control, Group C (335.83±156.92) vs Control. Estrogen was not significantly changed in Group A and Group B but increased in Group C than controls. 78.9% males in Group A, 72.2% in Group B, 12.5 % in Group C and 7.5% in controls had progesterone levels less than 0.28 ng/ml. Significant positive correlation was seen between BMI and CD4, CD4 and testosterone, BMI and Estrogen.We conclude that hypogonadism is common in HIV patients which need further studies to find the cause of hypogonadism as early diagnosis can help in better management of HIV patients.

P085

Evaluation of the Thyroid Profile Status Among Pregnant Women in their early Pregnancy in East Sikkim

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Thyroid dysfunction during pregnancy can lead to severe maternal and fetal adverse outcome and it is the second most common endocrine disorder during pregnancy. Iodine deficiency disorder can lead to both hypo and hyperthyroidism. Sikkim is a north eastern state lying in the Himalayan goiter belt which is predominantly due to iodine deficiency. Therefore, it was felt necessary to evaluate the thyroid profile status among pregnant women in Sikkim.The objective of this study was to evaluate the thyroid profile status in early pregnancy in Sikkim. This was a hospital based, observational cross sectional study. 208 women in their early pregnancy were selected and their thyroid function tests were done for all consenting participants. History and demographic profile of the subjects was noted. Total 208 pregnant women were evaluated for thyroid profile status. 7.2% of total (15) were found to have subclinical hypothyroidism of which 4.3% women were in the 1st trimester and 2.9% were in the 2nd trimester. 1.4% of the pregnant women were hypothyroid. Among the pregnant women 3.4% were hyperthyroid with 2.9% among them being in the 1s trimester of pregnancy. Hypothyroidism both subclinical and overt is a problem in Sikkim and surprisingly the number of pregnant women with hyperthyroidism was also higher than expected but consistent with findings of few other studies. Thyroid dysfunction was higher in the 1st trimester in comparison to the 2nd trimester.

P086

Association of Liver Enzymes in Type 2 Diabetes Mellitus with Hyperlipidemia

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Diabetes mellitus is a metabolic disease known by chronic hyperglycemia which results from defective insulin action and secretion. Altered lipoprotein pattern and liver enzymes have been identified as independent risk factors for the development of cardiovascular disease (CVD). Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia and is associated with dyslipidemia and disturbed liver function. Aim of the present study was to assess the liver enzymes and to find its association with

hyperlipidemic profile in T2DM. Total of 100 subjects were studied and divided into two groups; diabetes (n=50) and non-diabetes (n=50). Various biochemical parameters like fasting glucose, post prandial glucose, total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL), alanine amino transferase (ALT), and aspartate amino transferase (AST) were analyzed by ERBA Autoanalyser. Low density lipoprotein cholesterol (LDL) was estimated by Freidwald's formula. Statistical analysis was performed by applying student t test and Pearson's correlation coefficient and the results were fund to be significant (P value < 0.05). All the glycemic control parameters, lipid profile parameters and liver enzymes were found increased in diabetes group and significantly differ from non-diabetes group (P<0.05). ALT and AST showed significant positive correlation with fasting glucose, post prandial glucose, TC, TG, and LDL at P<0.05 but negative correlation with HDL concentration.T2DM incline to elevate liver enzymes, especially AST and ALT were of significance. Routine screening of AST and ALT in T2DM patients may assists early detection of liver abnormalities and to arrest the progress of disease.

P087

Insulin Resistance: A Metabolic Link Between Freshly Detected Diabetes Mellitus and Psychiatric Morbidities

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Patient with diabetes mellitus shows an increase prevalence of various psychiatric disorders mainly depression and anxiety.Insulin resistance may trigger the production of counter-regulatory hormones that play a role in it. In patient with psychiatric illness the Hypothalamic – pituitary – adrenal axis in hyperactive. Excess circulating cortisol and its disruption of glucoregulatory mechanisms is thought to lead Hyperinsulinemia and insulin resistance.The purpose of this study was to find out the prevalence of psychiatric morbidities in patient with freshly detected diabetic patients and its relation with Insulin resistance. In this cross sectional study 100 patient aged between 30-60 yrs who fulfilled the criteria for diagnosis as per the WHO criteria of diabetes mellitus were selected randomly from endocrinology OPD of S.S. Hospital, Banaras Hindu University, Varanasi between September 2013 to August 2014. The patients were assessed for psychiatric morbidities as per the DSM-IV TR criteria and Insulin resistance from blood chemistry measure of fasting insulin (by ELISA KIT) and fasting glucose using Homeostatis model assessment method (HOMA –IR Scale). In the study group of 100 patients, 34% of the patients were found psychiatric illness, not taking any treatment. P value calculated by chi- square test for psychiatric illness and insulin resistance was 0.0139.This study predicts the prevalence of psychiatric illness in newly detected diabetes and hightlights the need of psychiatric evaluation at the time of diagnosis of diabetes mellitus and letter on. A positive association was found between insulin resistance and psychiatric morbidities in newly detected diabetes patients.

P088

Correlation of Serum Vitamin D_3 and Serum Calcium Levels in Type II Diabetes Mellitus

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Vitamin D_3 has been identified to play a role in the homeostasis of glucose metabolism and the development of both type 1 and type 2 Diabetes Mellitus. The association and possible interaction of the two factors- Serum Vitamin D_3 and serum calcium levels in type 2 Diabetics may play a significant role in the aetiology and maintenance of glycemic and nonglycemic targets. Besides, it would provide an insight and understanding of the metabolic factors responsible. Present study was a hospital based cross sectional study carried out in the Department of Medicine of Shri Mahant Indiresh Hospital, Patel Nagar, Dehradun over a period of two months and included patients who visited to the OPD/IPD of the Department. The study group included 36 patients suffering from type 2 Diabetes mellitus and 36 subjects in the control group which were selected from the healthy population who were non diabetic.The glycemic parameters considered in the present study were fasting blood sugar (FBS), post-prandial blood sugar (PPBS) and glycosylated haemoglobin (HbA1C). The majority of them had uncontrolled diabetes at the time of presentation as depicted by the glycemic parameters. The mean fasting blood sugar was 167±68 mg/dl (males: 164±85.6 mg/dl and females: 171.33±47.09 mg/dl). These were unmodified blood sugar as most of them had presented for the control of their blood sugar or had complications at presentation. The post-prandial blood sugar also showed an uncontrolled trend with mean blood sugar being 235.5±101.08 mg/dl as compared to the controls that showed 111.8±14.48 mg/dl as mean post-prandial blood sugar. The non-glycemic parameters in the study included Body Mass Index (BMI), 25 (OH) D_3 levels and serum calcium. The mean serum calcium levels in the study and control group was 9.74±0.6 and 8.8±0.42 mg/dl respectively. The mean 25(OH) D_3 levels in the study group was 12.37±3.6 mg/dl whereas in the control group, it was 20.65±13.07. This difference was significant in the two groups (P<0.05).Vitamin 25(OH)D_3 levels were significantly lower in diabetics as compared to non diabetics. Serum Calcium levels were significantly higher in diabetics as compared to non diabetics. There was a positive correlation of serum calcium and fasting blood sugar in diabetics. This correlation was significant with blood sugar levels above 200 mg/dl. With lower blood sugar levels, no correlation was found.

P089

Dyslipidemia in Subclinical Hypothyroidism

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Subclinical hypothyroidism (SH) has prevalence between 4% and 10.5% in various studies. The burden of SH in India is expected to increase with increasing iodine insufficiency. Sublinical Hypothyroidism leads to altered lipid profile according to previous studies.Aim is to assess the association of SH and lipid profile. Estimation of lipid profile (Cholesterol, Triglyceride and HDL) was done in subclinical hypothyroidism (50) and compared with the healthy control group (30). In the present study the mean Total Cholesterol values were 131.0±22.38 mg/dl, 172.06±27.51 mg/dl, serum triglyceride values were 113.83± 20.45 mg/dl, 169.67±31.24 mg/dl, LDL Cholesterol values were 66.06±23.29mg/dl, 99.10±27.43 mg/dl, serum HDL Cholesterol values were 42.36±4.17 mg/dl, 38.56±4.14 mg/dl, respectively in healthy control, subclinical hypothyroidism. Mean T. cholesterol, mean LDL and mean TG were significantly higher in SH (P<0.001) compared to controls. Mean HDL was also lower in SH. Difference in TSH levels were less significant. In subclinical hypothyroidism dyslipidemia is more common as compared to the controls and is TSH depenent.

P090

IMA and NO in Hyperthyroidism

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Increased ischemia modified albumin levels have been described as a marker of ischemia reperfusion injury and; dysfunction of the endothelial L-arginine/nitric oxide pathway is a common mechanism by which several cardiovascular risk factors mediate their deleterious effects on the vascular wall. No reports are available in literature to comment on the simultaneous measurement of these parameters in hyperthyroidism. Therefore this study was planned to evaluate the levels of ischemia modified albumin levels and nitric oxide in patients of hyperthyroidism.This study was conducted on 50 newly diagnosed hyperthyroid patients and the results were compared with 50 age and sex matched healthy controls. Ischemia modified albumin levels and nitric oxide were estimated by standard colorimetric methods. Nitric oxide concentration was found to be significantly low in hyperthyroid patients (6.4 ± 30.8 μmol/L) as compared to control subjects (36.24 ± 7.61 μmol/L); (P< 0.05) while ischemia modified albumin levels were found to be higher in hyperthyroid group (0.662 ± 0.17 ABU) than healthy controls (0.290 ± 0.09 ABU) (P< 0.05). Ischemia modified albumin levels were negatively correlated with nitric oxide (r = -0.761, P< 0.001) and correlation was highly significant. Increased ischemia modified albumin levels may be a consequence of and cause for decreased nitric oxide levels in hyperthyroidism and this study may help to establish the role of ischemia reperfusion in the pathogenesis of hyperthyroidism.

P091

Levels of Cardiac Troponin I Ultra in Healthy Neonates

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Troponins are regulatory globular proteins, primarily located on the myofibrils andreleased into the circulation on myocardial Injury. Human cardiac Troponin I (cTnI) is a 30 amino acid peptide longer than skeletal Troponin I giving it unique cardiac specificity. Only one cardiac isoform has been identified. Ischemia and myocardial necrosis occur in 25–51% of newborn infants with perinatal asphyxia and are often associated with other adverse conditions specific to the neonatal period. Thus estimating the levels of cTnI in normal neonates could be of significance in conditions of foetal distress. Aim was to estimate the levels of cTnI ultra in healthy neonates.It is a retrospective database study carried out at PGIMS Rohtak in 116 asymptomatic neonates with gestation age =37 weeks and APGAR score >7 at 5 minutes. Cord blood samples were analysed for cardiac troponin I ultra on ADVIA centaur CP by chemiluminiscent technology. The Mean cTnI ultra in the healthy neonates was found to be 0.0502 ng/ml. The mean APGAR score was 7.215 at 1 minute and 8.922 at 5 minutes. Keeping in view of small therapeutic window of intervention in newborns, cardiac Troponin I ultra may be a sensitive marker for early detection of neonatal morbidity.

P092

Lipoprotein A as a Predictor of Steroid Dependence in Idiopathic Nephrotic Syndrome in Children

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Serum Lipoprotein A (LpA) levels increase in nephrotic syndrome (NS). The majority of children with NS in India have the steroid sensitive variety, with high potential for final cure. However more than 60 % suffer from relapses, and about 30% have steroid dependence. This study evaluated the potential of LpA, measured on admission in the first NS episode, for the prediction of subsequent relapses or steroid dependence. Children (n=40) diagnosed with first episode N Son admission to a pediatric hospital were tested for LpA (mg/dl) and also had standard tests such as serum albumin, cholesterol, triglyceride, urine protein etc. on Roche Integra (Dec2011-Dec2012). Children were followed for a minimum of one year from diagnosis to record relapse episodes and steroid dependence. Patients were categorized as: no relapse (NR), infrequent relapse (IR), frequent relapse (FR) and steroid dependent (SD) as per standard definitions. 20 healthy volunteers were also tested for lipid profile and LpA levels. Of 40 cases (median age 3y, 23 males), based on response to steroid, the outcome was 15: NR, 6:IR, 2:FR and 14:SD. The median LpA of NS group [136(IQR 60-232)] was significantly higher compared to controls [32(14-49)](P<0.0001). All other lipid parameters except HDL-cholesterol were also significantly higher in the NS group. Within the NS group, LpA showed significant correlation (Spearman-rho) with serum albumin (P=0.0034) and protein (P=0.04) but showed no correlation with the lipid parameters or urine protein. Comparison of LpA in the NR, relapsers (IR & FR) and SD groups revealed that the SD patients had a high LpA [213 (119-245)] compared to NR [90(46-195)] (P=0.04) and also to relapsers [110 (37-222)](P=0.11). Concentration of plasma LpA in patients with SDNS was significantly higher than in patients who did not suffer any relapse, and this may serve as a marker for prediction of SDNS at NS onset.

P093

Evaluation of Serum Magnesium Level in Neonatal Hyperbilirubinemia

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Hyperbilirubinemia is one of the most common problem encountered in newborn. Elevated unconjugated bilirubin increases activation of NMDA receptor/ion channels (N-methyl-D-aspartate) in CNS by modifying its binding characteristics and thereby resulting in neuronal injury. Magnesium is an important antagonist of NMDA receptor. It protects CNS against hypoxia and exerts neuroprotective effect by blocking excitotoxic and NMDA receptor mediated neuronal injury. Many physiological functions of magnesium act against or compensate for neurotoxic effect of raised bilirubin level. Objective was to evaluate level of magnesium in neonatal non haemolytic hyperbilirubinemia and correlate it with the level of serum bilirubin.The present study include 200 term, appropriate for gestational age neonates (1 – 7days old) admitted in Pediatrics Dept., GMCH. 100 neonates with non haemolytic hyperbilirubinemia were taken as study group and 100 neonates without hyperbilirubinemia as control.Total serum magnesium level and bilirubin level by standard photometric method determined in both the groups and appropriate statistical tests(mean±SD, P-value, Pearsons correlation test) done to analyse the results.The results suggest significant increase in total serum magnesium level in study group (P=0.05) compared to control group and a positive correlation between serum magnesium and bilirubin level in neonates. The results of the study suggest that increased level of serum magnesium in hyperbilirubinemia cases might be due to extracellular movement of magnesium, a principal intracellular cation resulting from bilirubin induced generalized cellular injury including neurons and erythrocytes. Considering the neuroprotective functions of magnesium we may speculate the possibility of a neuroprotective role or a compensatory mechanism in raised magnesium against emerging toxicity risk of high unbound serum bilirubin level.

P094

Analysis of Relationship between HbA2 levels and Iron Deficiency in Beta Thalessemia Trait

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Beta-thalassemia is of public health importance in many parts of the world. Screening for β-thalassemia trait (BTT) is necessary for family counseling. The potential causes of "false-negative" testing must be understood (i.e., circumstances in which the individual being tested has BTT but HbA_2 is not elevated). One example is $(\alpha\beta)^0$-thalassemia in which there is decreased production of α-globin chains as a result of α-globin gene deletion Carriers of β^+-thalassemia mutations reportedly have lower HbA_2 than carriers of β^0-thalassemia mutations. There is a small subset of individuals with BTT and normal α-globin genes who have normal HbA_2 levels. Some reports have suggested a lower than expected HbA_2 in individuals with BTT who are iron-deficient. Other studies have found no significant effect of iron deficiency on the level of HbA_2 in BTT. These divergent reports have led to confusion about the reliability of this test in the presence of iron deficiency. Aim was to find the relationship of HbA2 with iron deficiency indicators. In this study, we evaluated 40 individuals who were recruited for a pilot study under NHRM project for β-thalassemia in Jharkhand. Subjects were documented to have BTT by HPLC (Variant 2 Bio Rad) and Cellulose Acetate electrophoresis (GmbH Germany). Serum ferritin measurements and hemoglobin analysis by HPLC were done, thus allowing us to analyze the possible relationship between low serum ferritin and HbA_2 level. Furthermore, we sought to evaluate other clinical and laboratory determinants that may be related to HbA_2. The mean HbA_2 (5.3%) in individuals with serum ferritin <14 μg/L was lower than those who are not iron-deficient (5.6%; $P = 0.002$). Nevertheless, HbA_2 in individuals with serum ferritin <14 μg/L ranged from 4.3 to 6.5%, with none <3.5%.variability in this population. Based on the results of this study, elevated HbA_2 is a reliable marker of BTT, even in the presence of iron deficiency. In patients suspected to have BTT, but in whom HbA_2 is found to be <3.5%, testing for α- and other globin gene abnormalities should be pursued.

P095

Detection of Glutaric Acidemia Type 1 in Infants by using Tandem Mass Spectrometry

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Glutaric acidemia type1 (GA1) an autosomal recessive disorder is caused by deficiency of the enzyme Glutaryl- Co A dehydrogenase. Only 500 cases have been reported worldwide. GA-I is characterized by an accumulation of glutarylcarnitine (C5DC) and glutaric acid (GA) measured by LC- MS/MS. Aim was to determine the sensitivity and specificity of the tandem mass spectrometer to detect cases of glutaric acidemia type1 in both apparently healthy newborn and high risk children. A prospective study where 17,100 newborns were screened for inborn errors of metabolism during a 3 year period and followed up. Dried blood spots were then analyzed by the LC-MS/MS (TMS) where amino acids and acylcarnitines were estimated. Samples were interpreted as normal and GA1 presumptive positive according to the cut off and clinical presentations. 14 of the total had a presumptive positive for GA1. The sensitivity and specificity of the screening tests was 93.33% and 99.42% respectively. Presumptive positive cases were further confirmed by second tier tests and started on treatment. Older age of diagnosis had poorer outcomes. Despite of the very rare incidence of this organic acidemia, the TMS was able to pick up as many as 14 positive cases in a 3 year period. Analysis of dried blood spots by tandem mass spectrometry is therefore a sensitive and specific way to detect apparently healthy as well as symptomatic cases of glutaric acidemia type1. This emphasizes the need for new born screening.

P096

Hypolipidemic effect of *Momordica charantia* Fruit Extract in Rabbits

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Objective is to evaluate the effect of aqueous extract from fruit of Momordica charantia (MC) on lipid profile in experimentally induced hyperlipidemia in rabbits. Albino rabbits (1.5-2.5 kg) of either sex were divided into four groups with six animals in each group. Group I received standard chow diet, group II received Momordica charantia fruit aqueous extract (100mg/kg, po), group III rabbits received high fat diet (HFD), group IV rabbits received high fat diet supplemented with MC fruit aqueous extract

(100mg/kg). After 14 weeks of experimental period, animals were fasted overnight and blood was taken for estimation of total cholesterol (TC), triglyceride (TG), high density cholesterol (HDL-C). LDL -C, VLDL-C and atherogenic indices were calculated. Results (Mean ± SEM) were analysed statistically using student's "t" test (unpaired). Increased in triglyceride and total cholesterol was observed in rabbits maintained on HFD. Administration of MC aqueous fruit extract significantly lowered the serum levels of TC, TG and MDA levels in group II rabbits as well as in group IV. However, in rabbits of group IV MF supplementation led to increase in levels of HDL-C. Administration of MC aqueous fruit extract produces hypolipidemic effect in normal as well as in hyperlipidemic rabbits.

P097

Hepatoprotective Effect of Aqueous Extract of Leaves of *Ficus infectoria,* in Rats Intoxicated with Ethanol

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Liver dysfunction is a major health problem. Herbal drugs have been in use for treatment of liver diseases. *Ficus infectoria*'Pilkhan in Hindi', is a plant of great medicinal value. Objective was to evaluate the hepatoprotective activity of aqueous extract of leaves of *Ficus infectoria* (Aq.FI) in ethanol intoxicated rats. Rats were divided into six groups of six rats each. Group I healthy control, Group II, III, IV, V & VI were administered 20% ethanol (5gm /kg bwt./ day) orally once daily for 60 days. After 30 days Group III, IV and V received Aq.Fi at a dose of 200, 400 and 600 mg/kg, Group VI received Silymarin 100 mg/kg bwt. Biochemical investigations-Aspartate transaminase (AST), Alanine transaminase(ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT). Malondialdehyde(MDA), Reduced glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT). There was significant rise in liver marker enzymes like AST, ALT, ALP and GGT in group II. Thirty days treatment with Aq.Fi prevented rise in levels of these enzymes in group III-V. There was significant rise in MDA and fall in GSH, SOD and Catalase indicating compromised antioxidant status in group II. Treatment with Aq.FI prevented rise in MDA & increased levels of GSH, SOD and Catalase significantly ($P< 0.001$). Maximum effective dose of Aq.FI was 400 mg/kg. Hepatoprotective effect observed with this dosewas comparable to standard hepatoprotective drug Silymarin.The results of the present study indicate that Aq.FI possess hepatoprotective effect which may be due to its antioxidant property.

P098

Antidiabetic Effect of Aqueous Extract of Leaves of *Ficus infectoria* in Streptozotocin Diabetic Rats

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Herbal drugs have been in use for treatment of diabetes. *Ficus infectoria* 'Pilkhan in Hindi', is a plant of great medicinal value. Objective was to evaluate the antidiabetic activity of aqueous extract of leaves of *Ficus infectoria* (Aq.FI) in streptozotocin diabetic rats. Rats were made diabetic by i.p. injection of streptozotocin (45 mg/kg). Hot aqueous extract was prepared by boiling crushed leaves with water & filtering it. Rats were divided into five groups of six rats each and treated with AqFI for one month orally once daily as follows: Group I- Healthy control, Group II – Diabetic control, Group III-IV & V Diabetic rats treated with AqFI 100, 200& 400 mg/kg/day, Group VI- were received glibenclamide (0.5 mg/kg). Biochemical investigations-fasting blood glucose (FBG), postprandial blood glucose (PPG), glycosylated hemoglobin (HbA1c), serum insulin, total cholesterol (TC), try acylglycerol (TAG), LDL+VLDL cholesterol (LDL-C+VLDL-C) and HDL cholesterol (HDL-C).There was significant fall in FBG & PPG in groups III, IV& V. Maximum fall was obtained with a dose of 200mg/kg. There was 44% fall in FBG and 49% fall in PPG with this dose ($P<0.0001$).Treatment with AqFI decreased Glycosylated hemoglobin and increased fasting serum insulin ($P< 0.0001$). Aq.FI improved lipid profile:- Decreased TC, TAG, LDL-C + VLDL-C while HDL-C increased ($P< 0.001$). Result with AqFI were comparable with result obtained with standard drug glibenclamide. The results of the present study indicate hot aqueous extract of leaves of *Ficus infectoria* have potent antidiabetic and hypolipidemic effect.

P099

Polar Metabolite Concentrate of *Saraca asoca* Flowers Down-regulates the Aerobic Respiration and Membrane Transport Systems in *Pseudomonas aeruginosa*

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Gram negative bacteria contribute to wide variety of infections with a characteristic tolerance towards a variety of environmental conditions and drug resistance. Objective was to explore the novel antimicrobial metabolites or compounds from natural sources and their mechanisms of action. In the current study, antimicrobial polar metabolite concentrate of the dried flowers of *Saraca asoca* was prepared and *Pseudomonas aeruginosa* was treated with sub-MIC of the extract. Bacteria grown under restrictive stress were studied for differential expressed proteins and polar metabolites using 2-dimentional gel electrophoresis and high performance chromatography coupled with quadruple time of flight mass spectrometer. Analysis of data showed the presence of 117 and 219 differentially regulated metabolites and proteins respectively. Extract disturbed the integrity of cell membrane which in turn disturbed the functions of membrane embedded proteins i.e. transporters and signaling proteins. Down-regulation of electron transport chain, *rhl* and *las* quorum sensing systems, transport systems like SdeX and T4SS led to several concurrent events and increase, ionic environment, osmotic pressure and oxidative stress in bacteria that ultimately kill bacteria.The present study not only gives the novel anti-bacterial mechanism of action but also opens a new paradigm for use of traditional plants to cure diseases even in modern time.

P100

Can Sleep Deprivation Predispose to Impaired Glucose Tolerance?

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Sleep deprivation has been linked to increased sympathetic nervous activity, which in turn can predispose to IGT thus possibly increasing the risk of developing Type II Diabetes Mellitus. Aim was to measure and grade sleep deprivation using Pittsburgh sleep quality index (PSQI) software in patients with depression. Aim was to estimate and compare blood glucose levels with sleep deprivation index in these patients and compares them with that of healthy controls.The study subjects (n= 57) with depression with sleep deprivation were recruited from department of psychiatry from Dr. A. V. Baliga Memorial hospital.The study involved an interview as well as a self-reported questionnaire and was scored using Pittsburgh sleep quality index (PSQI) software. Fasting blood glucose was estimated using glucometer. A significant increase in fasting sugar was observed in sleep deprived patients as compared to that of healthy controls ($P<0.001$). Correlation of Pittsburgh sleep quality index score with fasting sugar also will be discussed. Sleep deprivation can induce some stress which can predispose a person to impaired glucose tolerance.

P101

Inhibition of Angiotensin Converting Enzyme by Aqueous Extract of *Ocimum sanctum*

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Angiotensin I converting enzyme (ACE) is widely distributed and has several important physiological functions including its role in blood pressure regulation. ACE inhibitors are used in the treatment of congestive heart failure, coronary artery disease, diabetic nephropathy etc. The present study was aimed to find the effects of aqueous extract of *Ocimum sanctum* leaves on ACE present in pleural fluid. ACE activity in the pleural fluid was measured with Hippuryl-Histidyl-Leucine (HHL) as substrate and the Hippuric acid released was measured spectrophotometrically at 228 nm. Aliquots of aqueous extract of *Ocimum sanctum* leaves were used in the enzyme assay to determine its effect on ACE. The linearity of ACE activity was established with HHL for the incubation period of 30 minutes at 37°C. ACE activity was confirmed with specific ACE inhibitors like Captopril, Lisinopril, and Enalapril. ACE activity was determined in the presence and absence of 1:1 aqueous extract (w/v) prepared from *Ocimum sanctum leaves*, which inhibited ACE activity significantly. 25 µls of *Ocimum sanctum* reduced ACE activity by 40% compared to control. Use of medicinal plants is gaining considerable importance in the treatment of hypertension. Results of this study indicate the presence of a potential ACE inhibitor in the aqueous extract of *Ocimum sanctum* leaves.

P102

Mass Spectrometry: Future of Laboratory Diagnosis or Still A Dream

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While the accuracy of conventional analytical techniques such as photometry, potentiometry, and immunoassay in detecting clinically important substances is often limited. Methods based on mass spectrometry allow highly specific detection of target compounds at the level of their respective molecular structure. Review based on the authors' scientific and observed practical knowledge. Mass spectrometry has contributed significantly to the advance of medical science in recent decades, particularly in relation to drug development, in-vitro diagnostics, and nutritional and environmental medicine. Its role has widened significantly in recent years, thanks to the development of soft ionization techniques and complex ion analysis (in particular, LC-tandem mass spectrometry, and MALDI-TOF**).** With its variety of methods and applications, the basic technology of mass spectrometry represents a key technology in biomedical research. In numerous fields of in-vitro diagnostics mass spectrometry based methods have already enabled the introduction of innovative routine analyses.

P103

Breath Analysis by Residual Gas Analyzer-Mass Spectrometry: A Non-invasive Method for Screening Individuals with Prediabetes and Type 2 Diabetes

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The pre-diabetes (PD) and type 2 diabetes (T2D) are diagnosed by means of oral glucose tolerance test (OGTT), glycosylated hemoglobin (HbA1c) test etc. These methods are invasive and need blood samples.The aim of the present study was to diagnose pre-diabetes and type 2 diabetes from breath analysis by utilizing a simple residual gas analyzer mass spectroscopy (RGA-MS) coupled with an ultra-high vacuum chamber. After administration of non-radioactive ^{13}C-glucose (75 mg) along with 75 gm normal glucose, breath samples were collected at baseline and 0.5h intervals for upto 5h. The breath samples were analyzed by RGA-MS system to record the ion currents of different masses in the exhaled breaths. From the ion-current measurements of several masses by RGA-MS technique, we found the least enrichment of glucose derived breath $^{13}CO_2/^{12}CO_2$ isotope ratios (45 and 44 masses), expressed as $\alpha_{DOB}{}^{13}C‰$, for type 2 diabetes followed by pre-diabetes and controls. The optimal diagnostic cut-off points were determined to be $\alpha_{DOB}{}^{13}C‰ = 28.32‰$ and 19.32‰ for screening individuals with control vs pre-diabetes and pre-diabetes vs type 2 diabetes respectively. Further, we also observed significant ($P<0.05$) changes in the ion currents of additional few masses (12, 15, 21, 41, 42, 33) from baseline samples in the breath of control, pre-diabetes and type 2 diabetes by RGA-MS analysis. The changes in ion-currents of these masses during respiration may be associated with the metabolic defect of early stage and type 2 diabetes during OGTT. Our observations suggest that a simple RGA-MS may serve as an alternative non-invasive point-of-care diagnostic tool for routine clinical practices as well as for large-scale diabetes screening purposes in real-time.

P104

Study of TSH Level in Patients with Metabolic Syndrome

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Metabolic syndrome consists of constellation of metabolic abnormalities which includes central obesity, hyperglycemia, high triglyceride, low high density lipoprotein cholesterol and hypertension. According to International Diabetes Federation (IDF), obesity is a key component of metabolic syndrome which occurs due to increased energy intake, decrease energy expenditure or combination of both thus leading to positive energy balance. Thyroid hormone up-regulate metabolic pathways relevant to resting energy expenditure, hence, obesity and thyroid function are often correlated. Objective was to determine the relationship between serum level of Thyroid stimulating hormone and metabolic syndrome. Data was collected from patients attending medicine OPD in RMCH, Bareilly. 30 cases (3 out of 5 criteria by IDF) and 30 aged matched controls were included in the study after full filling the inclusion and exclusion criteria. Appropriate statistical analysis was done using SPSS software. P value less than 0.05 was considered significant. Patients with metabolic syndrome had significantly high level of TSH. Metabolic syndrome and hypothyroidism are independent risk factors for the same disease process namely dyslipidemia. Patients with metabolic syndrome are at a risk of developing hypothyroidism. Both these diseases entities have a compounded risk of developing cardiovascular disease.

P105

A Comparative Study of SHBG, Testosterone and Insulin Resistance in Obese and Non-Obese PCOS Women

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Obesity is very common clinical feature in women affected by PCOS. In fact 30-75% of PCOS women are overweight or obese. In Combination with elevated ovarian production of androgen, the amount of circulating sex hormone binding globulin (SHBG) also plays a role in pathogenesis. There is inverse relationship between insulin and SHBG. Insulin inhibits hepatic production of SHBG. The decrease in SHBG produced by hyperinsulinism in PCOS obese underlies the decrease in total testosterone, since SHBG is its main transporter. This study was designed to compare the biological variability of total testosterone, SHBG and insulin resistance in obese women and non-obese PCOS woman. Further to evaluate SHBG, a predictive marker for IR in PCOS woman. Serum blood samples were collected from 120 PCOS obese (n=60) and non-obese women (n=60) with age (20-40years). Samples were analyzed for SHBG, Total testosterone; FSH, LH and estradiolwere measured with Enzyme-linked immunosorbent assay (ELISA) and serum levels of insulin, glucose, insulin glucose ratios, and the free androgen index were determined and compared in obese and non-obese PCOS woman.The biometric and biochemical parameters were determined in PCOS obese and non-obese woman. The biometric and biochemical parameters show a significant difference between obese and non-obese PCOS woman. Whereas SHBG and total testosterone were significantly lower in PCOS obese group compared to non-obese individuals. Collectively, further clinical studies will be warranted to determine that in PCOS obese woman may show a low SHBG concentration reflects an elevation in IR. When SHBG variability was less, the variability of testosterone was also reduced compared to PCOS non-obese woman. Our findings support earlier observations that an important association exists between the SHBG levels, testosterone and insulin resistance in PCOS obese women. In addition SHBG serves as a predictive marker of IR in PCOS woman, particularly in those who are obese.

P106

Microalbumiuria as a Marker of Renal Complications in Type 2 Diabetes Mellitus

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Diabetes mellitus is a stage of persistent hyperglycemia due to absolute or relative deficiency of insulin. About 20 – 40% of patients with type 2 diabetes mellitus develop diabetic nephropathy. The earliest clinically detectable sign of nephopathy is microalbuminuria. Microalbuminuria is defined as urinary excretion of albumin of 20 – 200 μg/min or 30 – 300 mg/ 24 hrs or 30 – 300 mg/ g of creatinine with a negative dipstick test. Prevalence of microalbuminuria and its association with various biochemical parameters. The study was carried out on 100 controls and 100 known cases of type 2 diabetes mellitus of duration more than six month in Biochemistry Department. Patient' s age, gender, duration of diabetes, levels of fasting plasma glucose (FPG), serum urea, serum creatinine and glycosylated hemoglobin (HbA1c) were estimated. The prevalence of microalbuminuria in our present study was found to be 48%. The patients with higher HbA1c and FPG level had high incidence of microalbuminuria as compare to controls ($P<0.001$). Diabetics patients with microalbuminuria had higher levels of serum creatinine ($P<0.05$) and serum urea ($P<0.001$) as compare to controls. Uncontrolled diabetes mellitus patients had high incidence of microalbumiuria. As microalbuminuria is an early marker of diabetic nephropathy, it is possible to halt or delay the progression of diabetic nephropathy at this stageby the use of appropriate treatment modalities such as angiotensin converting enzyme inhibitors. The study will be helpful thereby in reducing morbidity and increasing life expectancy of patients with type 2 diabetes mellitus.

P107

A Clinical Study to Evaluate the Hypoglycemic, Antidyslipidemic and Antioxidant Activities of *Tinospora cordifolia* in Management of Type - 2 Diabetes Mellitus

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Tinospora cordifolia (Menispermaceae) is widely used in Ayurvedic medicine as a remedy for metabolic disorders however, anti-diabetic and anti-dyslipoproteinemic activities are not well studied in diabetics this study is carried out to explore these effects in diabetic type 2 patients. Aim was to explore anti-diabetic, anti-dyslipoproteinemic and antioxidant activities of *Tinospora cordifolia* (Menispermaceae) in type 2 diabetic patients. In this study clinical trial was conducted on type 2 diabetic patients attending diabetes OPD, Kaya Chikitsha Vibhag, Rajkiya 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 plasma levels of fasting blood sugar, lipid profile accompanied with increase in the lipids and apo-protein levels of serum β lipoproteins (very low density lipoprotein; VLDL and low density lipoprotein; LDL) following decrease in lipid and protein constituents of α lipoprotein (high density lipoprotein; HDL) 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 in type 2 diabetic patients was examined. Outcome, significant decrease in the level of blood sugar fasting, total cholesterol, β lipoproteins and triglyceride. The decrease in lipids and apoprotein levels of β lipoproteins was accompanied with stimulation of plasma lecithin cholesterol acyltransferase (LCAT). Lipid and apoprotein level of α lipoproteins also partially recovered. Results of this study lead to research and development of a potent anti diabetic antidyslipoproteinemic drug from *Tinospora cordifolia* stem.

P108

Study on Correlation of Lipid Profile Among Hypothyroidism and Type II Diabetes Mellitus

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Dyslipidemia is common metabolic abnormality in thyroid disorders overt or subclinical and diabetes mellitus with marked increase in circulating low density lipoprotein (LDL-C). Several studies have demonstrated significant variations in dyslipidemic patterns in Type II Diabetes Mellitus with Hypothyroidism.The study was carried out to know the prevalence of long term complications in relation to the lipid profile in patients having hypothyroidism and Type II Diabetes Mellitus. The study was carried out on patient attending in Katihar Medical College & Hospital from June 2012 to April 2014. Serum TSH, fT3, fT4 was estimated by Chemiluminence Immunoassay system. Plasma glucose was estimated by GOD-POD technique.Total cholesterol,Triacylglycerol, High density lipoproteins was estimated by CHOD-PAP technique, GPO-ESPAS technique, PEG-PAP method respectively. LDL was calculated by using the Friedewald formula.The lipid profile of only diabetic subjects showed TC (316.18±4.299 mg/dl), TG (358.36±5.544 mg/dl), LDL (214.70±4.192 mg/dl) with decreased HDL (29.80±0.348 mg/dl). The lipid profile in only hypothyroid patients showed TC (314.38±1.739 mg/dl), TG (322.46±2.429 mg/dl), LDL (208.69±1.665 mg/dl), HDL (41.20±0.3647 mg/dl). In subjects having both diabetes and hypothyroidism had TC (337.92±4.793 mg/dl) TG(350.02±5.127 mg/dl), LDL (236.17±4.093 mg/dl) HDL (31.74±0.285 mg/dl). LDL value was markedly high in diabetic hypothyroid patients. All the lipid profile parameters were significantly increased except HDL among the diabetics and hypothyroid subjects. Increase was more in cholesterol and LDL values among subjects suffering from both diabetes and hypothyroidism. HDL levels were lowest among the diabetics and also decreased among the diabetic hypothyroids.

P109

Reno-Protective Efficacy and Modulation of Advanced Glycation End Products by ACE 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 microvascular complication of diabetes mellitus (DM). Diabetic nephropathy is an irreversible process and cannot be completely cured. However, angiotensin converting enzyme (ACE) inhibitor therapy is believed to have some beneficial effects and is still under investigation. Hyperglycemia mediated advanced glycation end products (AGE) formation is the one of the major factor leading to diabetic complications.The present study was designed to explore the reno-protective efficacy of ACE inhibitor therapy in type 2 diabetic patients with nephropathy by following serum creatinine, urinary ACR, eGFR and serum AGE level. Forty eight patients with type 2 diabetes mellitus with evidence of persistent microalbuminuria (ACR; 30-300 mg/ g creatinine) or overt albuminuria (ACR; <300 mg/ g creatinine) tested on two separate occasions and belonging in CKD stage 1 to 3 were enrolled in this study. All enrolled patients were started on daily oral treatment with ramipril (dose titration 5mg to 20 mg). Follow up of all patients were done at 3 months interval for a total period of 12 months. 3 ml blood samples and morning spot urine samples were collected for analysis. Serum creatinine and urine creatinine were measured by alkaline picrate kinetic method. Urine microalbumin was estimated by nephelometry. Estimated glomerular filtration rate (eGFR) was calculated by following modification of Diet in Renal Disease (MDRD) equation and CKD-EPI equation. Serum AGE level was determined spectrofluorometrically. The results of present study indicate that ACE inhibitor therapy to diabetic nephropathy patients resulted in significant improvement in renal function as measured by serum creatinine, urinary ACR and eGFR. While there is approximately 21%, 49% decrease were recorded in serum creatinine and urinary ACR, about 28% increase was observed in eGFR. Serum AGEs level which is a marker of vascular complication in diabetes mellitus also decreased significantly on ACE inhibitor therapy during 12 months follow up. ACE inhibitor therapy have shown beneficial effects towards improvement of renal function and also significant reduction in AGE level indicating reduced possibility of development of diabetic complications.

P110

Association of Serum Magnesium Deficiency with Insulin Resistance in Type 2 Diabetes Mellitus

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Insulin resistance is the key pathophysiological defect that leads to development of type 2 diabetes mellitus. The purpose of this study was to estimate serum magnesium level and insulin sensitivity indices among type2 diabetes mellitus patients, and to see association between them. Study was carried out among thirty-eight type 2 diabetic patients and forty age and sex matched controls. Serum fasting glucose, magnesium, insulin, urea, and creatinine levels were estimated. Insulin sensitivity indices, homeostasis model assessment for insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) levels were calculated as per formulae. A highly significant low serum magnesium level was found in diabetic subjects as compared to the controls. Statistically significant high HOMA levels (> 2.6) and low QUICKI levels (< 0.33) were found among the case group. An inverse, statistically significant correlation was found between serum magnesium and fasting insulin level. A highly statistically significant inverse correlation was found between serum magnesium and HOMA level and a positive correlation was found between serum magnesium and QUICKI level, i.e. serum magnesium level decreases with increase in insulin resistance. A strong association was also found between fasting serum insulin level and insulin sensitivity indices. This study showed a lower serum magnesium level in diabetic patients compared to control. A strong association was also found between serum magnesium level and insulin sensitivity indices. For proper management of type 2 diabetes it may therefore be necessary to treat hypomagnesaemia in these patients.

P111

Identification of Metabolic Syndrome in Obese and Non-Obese Persons of Rural Population in Rajasthan

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In obesity, excess body fat accumulates to the extent that it may have an adverse effect on health, particularly heart disease, type 2 diabetes, breathing difficulties and osteoarthritis. Metabolic

Syndrome is considered to be an abnormality of obese persons and numerous studies have reported this association but very little data is available about Metabolic Syndrome in non-obese persons residing in rural areas. The study was designed to find out if, "No-Obesity means No-Metabolic Syndrome" in adults from a population of rural background. 100 adult subjects, both male and female were selected from the rural population attending the OPD of NIMS Medical College and Hospital. They were divided into 2 groups, Non-Obese and Obese, on the basis of established criteria. The selected subjects were further studied for the presence or absence of Metabolic Syndrome by estimating the biochemical correlates as per the NCEP ATP-III criteria. Student's 't' test was used to compare the biochemical correlates of various groups. Of the total obese subjects, about 16.2% were found to have Metabolic Syndrome. Moreover, of the total non-obese subjects, around 10.6% were found prone to Metabolic Syndrome. This alarming result reflects metabolic imbalances in rural adults due to the undergoing changes in their lifestyle. Thus, emphasis should be given to monitor the various parameters of Metabolic Syndrome even in rural population, so that these imbalances can be prevented at the earliest, as they affect the clinical and social setup of the society.

P112

Correlation Between Serum Electrolytes with Fasting Blood Glucose & HbA_{1C} Level in Indian Diabetic Population

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In diabetics, there is impaired insulin action causing alterations in serum sodium and potassium concentrations. The elevated sugar levels cause osmotic affects which along with cationic imbalance, could influence the course of diabetes mellitus management. Aim was to study the correlation between fasting blood glucose (FBS) & glycosylated hemoglobin levels with those of serum electrolytes including serum chloride levels. After a detailed history and examination blood samples were collected and assessed for FBS, HbA_{1C} and serum electrolytes ($Na+$, K^+, Cl^-) using different automated methods. 55 patients (M: F = 33:22) with mean age of 48.5 years were evaluated. 16 patients had type 1 DM who were on insulin, 35 had type 2 DM dependent on oral hypoglycemic agents (OHA) while 4 patients were on a combination of OHA & insulin. When we tried to correlate the values of serum electrolytes with FBS &HbA_{1C} levels, the P value was significant for correlation of serum Na^+ decreases with both FBS &HbA_{1C} (P = 0.02 & 0.049 respectively, insignificant for serum K^+ (P= 0.63 & 0.49), and significant for serum Cl^- with FBS (P =0.004) but insignificant with HbA_{1C} levels (P = 0.32). So we conclude that serum sodium levels are reduced in patients with diabetes mellitus while serum potassium has no correlation with its metabolic levels and serum chloride levels are related with FBS levels but not with HbA_{1C} levels.

P113

Role of C-Reactive Protein in Development of Hypertension

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Hypertension is one of the most important risk factor for cardiovascular disease and has become an increasingly important contributor to the global health burden. Inflammation and mounting of inflammatory markers (acute phase reactants) are important pathogenic mechanism in development of hypertension. It could be either a causative factor in the pathogenesis of hypertension or a sequel to it. In the view of the probable involvement of the acute phase reactants, present study was planned to investigate role of C-reactive protein (CRP) in development of hypertension and find out its correlation with blood pressure if any exist. For this study subjects (males) between 30-60 years of age were selected from the general population of Kota district (Rajasthan, India) and screened for hypertension. The study was done at MBS hospital & New Medical College & Hospital, Kota. CRP levels in the serum of 50 hyper-tensive cases and 50 healthy sex and age matched control subjects were determined on TRANSASIA EM-360 auto-analyzer using standard protocols. Results were expressed as Mean ± SD. Data were analyzed with the help of Microsoft excel 2007, using student's t-test, and strength of association between two variables is measured by Pearson's correlation coefficient (r). The mean serum CRP levels were significantly higher in cases as compared to control group (17.27±12.24mg/l versus 8.28±5.78mg/l, P<0.0001). CRP level showed a significant positive correlation with systolic blood pressure (P<0.001). Therefore, mounting of inflammatory markers is an important pathogenic phenomenon inhypertension. So appropriate measures should be taken to bring the separameters in limit for better prognosis and to avoid ill effects of hypertension.

P114

Estimation of Serum Triglyceride Level in Patients with Diabetes Mellitus and it's Correlation with Insulin Resistance: A Marker of Metabolic Syndrome

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Metabolic syndrome is characterised by abdominal obesity, hypertension, diabetes mellitus, insulin resistance, dyslipidemia and hyperuricemia.The most accepted pathophysiology of the metabolic syndrome is insulin resistance; hypertriglyceridemia is an excellent marker of the insulin resistance. Aim was to estimate triglyceride level in patients with diabetes mellitus and to establish its correlation with insulin resistance. 50 patients of type 2 diabetes mellitus fulfilling the diagnostic criteria (fasting and 2 hour post prandial blood sugar) and 50 healthy controls were in study. Serum triglyceride levels were estimated after overnight fasting by enzymatic method. After overall study the prevalence of insulin resistance was high (72%) among diabetic patients. 80 percent of diabetic patients had high serum triglyceride levels while only 22 percent of healthy controls had high serum triglyceride levels (P=0.007). Mean serum triglyceride levels in diabetic patients were significantly high as compared to healthy controls (P=0.009). High serum triglyceride levels had a positive correlation with insulin resistance.There was a higher serum triglyceride levels in patients with diabetes mellitus as compared to healthy controls. Hypertriglyceridemia is an excellent indicator of insulin resistance which is a marker of metabolic syndrome.

P115

Comparative Study of Level of Serum Uric Acid in Type 2 Diabetes Mellitus Associated with Hypertension

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The role of uric acid in the progression of prediabetes to diabetes has been known. Serum uric acid has been shown to be associated with cardiovascular disease, hypertension, and chronic kidney disease. However, conflicting data exist as regards the serum uric acid (UA) levels in type 2 diabetes mellitus, which are associated with risk factors and complications.The present study was designed to look for any association of serum uric acid with hypertension in type 2 diabetes mellitus, taking into consideration the relevant clinical, biochemical and the anthropometric data. Fifty patients with type 2 diabetes mellitus and 50 healthy controls were included in this study. They were further divided into different groups, based on the sex, the duration of diabetes, and the diabetes which was complicated with hypertension.The circulatory levels of glucose, total cholesterol and triglycerides were found to be elevated in the diabetics of either sex as compared to those in the controls. There was no significant difference in the serum uric acid levels between the diabetics and the non-diabetics, either in males or females. A negative correlation was observed between the fasting plasma glucose and the serum uric acid levels in both male [r = -0.60] and female [r = -0.60] diabetic patients. The serum uric acid levels marginally decreased with an increased duration of diabetes. The hypertensive male and female diabetics were found to have significantly decreased (P<0.05) serum uric acid levels as compared to the corresponding non-hypertensive diabetics. It was concluded from the present study that there occurs a significant decrease in the serum uric acid levels in hypertensive diabetics (both in males and females) in comparison with the non-hypertensive diabetics.

P116

Assessment of Lp (a) levels in Diabetes Mellitus and Study of it's Association with Glycemic Control and Lipid Profile

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The risk of cardiovascular disease is increased approximately two to four fold in patients with diabetes mellitus compared with non-diabetic individuals. The serum level of Lp(a) is an independent indicator of risk of development of vascular disease. Although numerous studies in the non-diabetic population have demonstrated an association between elevated plasma Lp(a) concentration and risk for atherosclerotic disorders, the contribution of Lp(a) to the enhanced risk of vascular disease in the diabetic population is not clearly defined and data in Asian Indian Diabetics is meager. Aim was to determine Lp(a) levels in Diabetics and non-Diabetic control group and evaluate Lp (a) concentration in relation to glycemic control and lipid parameters in Diabetic group. Diabetic patients (n=51) coming to the hospital OPD were chosen randomly over a period of 2 months. Healthy, non-diabetic controls (n=31) were taken from hospital staff. Serum Lp (a) was estimated by immunoturbidimetric assay, along with Serum Cholesterol, Serum Triglycerides and HDL on AU 480 auto analyzer. Appropriate statistical tests were applied. Lipoprotein (a) levels in Diabetics (n=51) was 27.9 $\pm$ 20.8 and in controls (n=31) was 28.2 $\pm$ 17.0 (P=0.93). Serum Cholesterol and Triglycerides was significantly higher in Diabetics (P<0.05), although no significant difference was observed in HDL value (P=0.35). Diabetic population was divided according to glycemic control, (HbA1c less than 7%, n=18) and

(HbA1c of 7% and above, n=33). No significant difference was observed in Lp(a) and lipid profile levels in these two sub-groups. Serum Lp(a) levels are not correlated to dyslipidemia and glycemic control in Diabetes Mellitus. It hence forth points to an independent mechanism of cardiovascular damage in DM.

P117

A Comparative Study of Serum HbA_{1C} In Obese Type II Diabetics with Obese Non Diabetics and it's Outcome in Malwa Region (M.P)

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Diabetes mellitus is the most prevalent metabolic & non-communicable disorder in the world. Around the world 200 million people have diabetes & it is predicted to increase to 300 million by 2020. The incidence of obesity as well as type II DM have shown alarming upward trends among Indian population & 80% of diabetics are overweight or obese. Serum HbA_{1C} is a routinely used marker for long-term glycemic control. This study is an attempt to evaluate the diagnostic value of serum HbA_{1C} in predicting diabetic dyslipidemia. Aim was to study serum HbA_{1C} in obese type 2 diabetics in comparison with obese non diabetics & its outcome in both the genders. Present case control study was carried out in collaboration of Department of Biochemistry & Medicine in Sri Aurobindo Medical College & P.G Institute Indore (M.P) in which 200 obese patients are grouped as case (type II diabetic) & control (type II non diabetic) by using their clinical & laboratory examination. In the present study, the results obtained are in cases (obese + type II DM) values of serum HbA_{1C} are much higher as compared to controls with significant increase in incidence of CVD, CVA and Hypertension. From the study we concluded that serum HbA_{1C} in obese type II diabetics as compared to obese non diabetics was significantly altered resulting into more morbidity in obese diabetics. Screening & early identification of disease can prevent morbidity and mortality in these groups of patient.

P118

Status of Interlukin-6 (IL-6) and Lipid Profile Levels in Depression Disorder

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Depression is one of the most common mental disorders, which is a mood disorder that causes a persistent feeling of sadness and loss of interest called major depression. It affects how you feel, think & behave and can lead to emotional and physical problems. Interlukin-6 is important biochemical markers involved are wide range in depression disorder. IL-6 is not only synthesized & released by the immune cells but also by cells in the CNS. The aim of this study was to find out association of the plasma concentration of IL-6 and lipid profile in the patients with depressive disorders and compare with healthy control group. This study is across –sectional, observational was conducted at GMCH, UDAIPUR in 50 patients having psychiatric disorder attending the psychiatric OPD, 50 normal healthy controls of both genders ranging in age from 20 to 65 years were selected for comparison with patients. There blood sample was collected and analyzed for Plasma IL-6 levels by ELISA method and lipid profile which includes serum total cholesterol, TG, HDL-C, LDL-C on fully automated serum chemistry analyzer Cobas- C 311. Suitable statistical analysis was done for evaluation of results. The Plasma levels of Total Cholesterol, Triglyceride, LDL -C , IL-6 was found significantly high and while High density lipoprotein (HDL-C) level was significantly low in psychiatric patients when compared with control healthy group. From this study we conclude that depression disorder are associated with lower HDL and higher TC, TG, LDL-C and IL-6 levels as compare to control group.

P119

Assessment of Cardiovascular Risk in Subjects with Metabolic Syndrome having Different Vitamin D Status – A Pilot study

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Metabolic syndrome (MS) is the leading determinant of the cardiovascular disease (CVD), which comprises one of the major causes of morbidity and mortality worldwide, and it may worsen further when the Vitamin D level of the people is decreased. Vitamin D insufficiency may influence the progression of the CVD in subjects with MS and the biomarkers have a role in diagnosis and prediction of events that lead to CVD. Aim was to assess the cardiovascular risks in subjects with metabolic syndrome (MS) among people with different Vitamin D status. It is a Case control study; test population comprises 30 patients who have been diagnosed to have MS. Detailed clinical, epidemiological and anthropometric characteristics were recorded using proforma. 5 ml of fasting venous blood collected from all the subjects after getting the informed consent, as per the criteria laid down by the Institutional Ethics Committee. The blood samples collected were processed and analyzed at fully automated central laboratory. In the current study lower levels of vitamin D was significantly associated with all the components of metabolic syndrome. Positive

correlation was found between glycemic parameters and metabolic syndrome. There was a significant increase in CRP level. Fibrinogen level correlates significantly with most of the components of metabolic syndrome. Hyperfibrinogenemia and an increase in CRP level in the test population explain the increased cardiovascular risk. The findings suggest an increase in cardiovascular risk in metabolic syndrome patients having vitamin D deficiency.

P120

Study of HDL-Cholesterol:LDL-Cholesterol in Hypothyroidism Patients

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Thyroid hormones effect metabolic processes in the body. Thyroid hormones significantly affect lipoprotein metabolism and thus abnormal thyroid function is suggested to have a causative role in cardiovascular disease. Objective was to evaluate HDL-cholesterol:LDL-cholesterol ratio in hypothyroid patients and to compare it with age and sex matched healthy controls.This was a cross sectional study conducted in the Department of Biochemistry, Pt B D Sharma, PGIMS, Rohtak. Thirty cases of hypothyroidism (subclinical and overt) and thirty age and sex matched healthy controls (euthyroid) were enrolled after informed consent and their fasting serum samples were analysed for serum triglyceride, total cholesterol and HDL cholesterol levels by enzymatic methods in autoanalyser and LDL cholesterol was calculated (Friedwald's formula) and HDL- cholesterol:LDL-cholesterol ratio was calculated. The mean values of HDL- cholesterol:LDL-cholesterol in hypoyhyroid patients was significantly lower as compared to that in controls with $P<0.05$. This study demonstrates that thyroid hormones may have an important effect on lipid profile. A lower HDL cholesterol:LDL-cholesterol ratio in hypothyroid cases also suggests that hypothyroid patients are more prone to atherosclerosis and cardiovascular disease. Measures to normalize thyroid levels in these patients have a preventive role in premature atherosclerosis. Altered ratio in subclinical patients suggests a need to undergo thyroid function tests (TSH, T_3 and T_4) in persons showing deranged lipid profile.

P121

Association of Uric acid Levels with Ischemic Stroke and Infarct Size in Men

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Stroke is one of the leading causes of mortality and morbidity worldwide, with ischemic stroke accounting for the larger percentage of total incidence. An intriguing aspect of stroke is its greater incidence in men than women, suggesting that male sex is an important risk factor. The levels of uric acid are higher in men as compared to women. Studies regarding role of uric acid in cerebrovascular diseases have produced inconsistent results so far. Study the levels of uric acid in men with acute ischemic stroke and correlate with infarct size. 50 male patients of acute ischemic stroke admitted in PGIMS, Rohtak and 50 age matched healthy controls were included in the study. Routine biochemical parameters were assessed in serum obtained from 5 ml of fasting blood sample. Patients with kidney or liver diseases, malignancies, diuretic use and alcohol intake were excluded. CT scan of brain was performed on patient's admission to hospital. The infarct size was measured in millimeters as the largest visible diameter of the infarct on CT. Serum uric acid levels were very significantly higher in cases ($P<0.001$) than controls. There was strong positive correlation between uric acid levels and infarct size on CT ($P=0.000$, $r=0.849$) and significant negative correlation between serum uric acid and HDL levels ($P=0.000$, $r=-0.653$). Additionally, infarct size correlated positively with triglyceride ($P=0.028$,$r=0.401$) and VLDL ($P=0.041$, $r=0.376$) and negatively with HDL ($P=0.000$, $r=-0.654$). The results suggest a significant association between uric acid levels and ischemic stroke in men which could be due to the free radicals generated during uric acid formation and/or due to the detrimental effect on endothelial and vascular function.

P122

Study of Thyroid Dysfunction in Type -2 Diabetes Mellitus patients in Agra city

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Diabetes mellitus (DM), a common endocrine metabolic disorder, is a leading cause of death worldwide. Thyroid disorders are also very common in the general population and it is second only to diabetes as the most common condition to affect the endocrine system. As a result it is common for an individual to be affected by both thyroid diseases and diabetes. In the present case control study, total 90 subjects were selected and divided into two

groups. Group 1 included 40 diagnosed cases with Type-2 DM and 50 age and sex matched healthy control subjects (Group 2). The fasting blood glucose, serum T_3, T_4 and TSH levels were measured in both groups. Blood glucose was estimated by GOD-POD enzymatic method. SerumT_3,T_4 and TSH levels were estimated by ELISA kit method. The levels of fasting blood sugar and serum TSH were significantly increased (P<0.001) in diabetic patients as compared to healthy control. However, no significant change was observed for serum T_3 and T_4 in the two groups. Estimation of T_3,T_4 and TSH is simple, reliable, economic and sensitive that can now be considered as an adjunct in the management of diabetes mellitus. Hence, the estimation of Thyroid hormone should be recommended in the panel of routine investigations for proper management of diabetes mellitus to prevent serious complications of the disease.

P123

Correlation Between Small Dense LDL and Insulin Resistance in Subjects Suffering from Cardiovascular Disease and Diabetes Mellitus

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Formation of small, dense LDL–C is closely associated with an increased cardiovascular risk, independently of the traditional risk factors. The formation of sdLDL is increased in the presence of insulin resistance and hypertriglyceridemia in diabetic patients.Our study aims to investigate the correlation between small dense LDL and insulin resistance in subjects suffering from cardiovascular diseases and diabetes.This study was carried out in Dept. of Biochemistry, G.R. Medical College, Gwalior. 46 clinically diagnosed subjects suffering from cardiac disorders and type II diabetes admitted in ICU of J.A. group of hospitals and 46 age and sex matched healthy control subjects were included in the study. We found significant increase in blood sugar (P<0.001), serum insulin (P<0.001), homa IR (P<0.001), TC (P<0.01), TG (P<0.001), LDL-C (P<0.001), VLDL-C (P<0.001), sdLDL-C (P<0.001) in CVD subjects compared to control group except HDL (P<0.001). Strong positive correlation was found between sdLDL-C and HOMA insulin resistance in the patient group (P<0.01). In conclusion, this study suggests that the formation of of small dense LDL-C is strongly correlated with insulin resistance. The increased insulin resistance in CVD subjects leads to the so-called atherogenic lipid triad: large numbers of sdLDL-C particles, hypertriglyceridemia, and low concentrations of HDL-C. Thus, people with insulin resistance have a characteristic dyslipidemia that has overproduction of VLDL and hypertriglyceridemia and in turn more formation of sdlDL-C which is autooxidizable and enhances the development of cardiac disorders.

P124

Thyroid Status and it's Correlation with Variations in Metabolic Parameters Leading to Other Diseased Condition

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Clinical hypo and hyperthyroidism is accompanied by variety of abnormalities in plasma lipids and glucose homeostasis leading to Diabetes Mellitus (DM) and Cardiovascular diseases (CVD), but unchecked status of subclinical (condition which is more common) for longer period may cause adverse clinical conditions. Presently there is no clear information on the correlation of DM and CVD with different stages of thyroid dysfunction. Thyroid dysfunction, (DM) and (CVD) are co-existent disorders and keeping in view the prevalence of thyroid dysfunction in individuals with DM and cardiac disorders the present study was planned to find a correlation if any amongst these abnormalities. We assessed the thyroid profile (T_3, T_4 & TSH) of 56 individuals with symptoms of DM and CVD divided as having normal thyroid function (group1), subclinical (group 3,5) and clinical (group 2,4) hyper and hypothyroidism respectively, based on the levels of TSH. The association of thyroid hormones was investigated with carbohydrate metabolism (levels of fasting serum glucose, insulin, C-peptide, HOMA-IR) and lipid metabolism [total cholesterol, TG's, HDL, LDL) & VLDL] in different groups. As compared to group 1, their was significant decrease in %S (P<0.001, P<0.05) and significant increase in IR (P<0.05) in Group 3 and 4 respectively with comparable IR in group 2 and 4. Levels of TG's were increased from (154.5±18.31 mg/dl) to (196.0±11.0 mg/dl) and (194.5±12.21 mg/dl) and TG's showed significant positive correlation with IR (r=+0.775 P<0.01, r=+0.643 P<0.001) in group 4 and 5 respectively. Also in group 1, 4 and 5 cholesterol showed significant positive correlation with LDL (P<0.001) and VLDL (P<0.05). Individuals in subclinical stages of thyroid dysfunction are at more risk for IR and its related disorders.

P125

Correlation of Serum Chromium, Zinc, Magnesium and SOD Levels with HbA_{1C} in Type 2 Diabetes: A Cross sectional Analysis

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The loss of dynamic integrity between homoeostasis of free radicals and antioxidants causes the development of complications like retinopathy, nephropathy, neuropathy, atherosclerosis and cardiovascular diseases in T2DM. Aim was to assess the concentrations of serum chromium, zinc, magnesium and SOD in subjects of T2DM and control and to investigate the effect of these variables versus HbA_{1C}. 50 Age & sex matched and normal glycaemic status subjects were taken into control group. 50 T2DM subjects were included in patient group. Micronutrients estimation was assessed by Atomic Absorption Spectrophotometer. Superoxide Dismutase was done by Marklund and Marklund modified by Nandi & Chatterjee method. HPLC was used to measure Glycated Haemoglobin Insignificant difference (P= 0.493) was reported in age (50 ± 4.7 year compared with 50 ± 7.2 year), while body mass Index (23 ± 2 kg/m^2 compared with 26 ± 4.5 kg/m^2) between the T2DM subjects and control subject showed significant difference (<0.0001). Inverse Pearson correlation coefficient, r (-0.376), (-0.689), (-0.05), (-0.05), (-0.40), (-0.14), (-0.342) and (-0.548) were established when HbA_{1C} of control and T2DM patients were compared with control and T2DM patients of serum Cr, Zn, Mg and SOD variables in that order. The overall "P" value demonstrated highly significant result at P< 0.0001 between the T2DM subjects and controls. Strong association between serum chromium and SOD in relation to HbA_{1C} in this study gives a strong point that these variables could be used as markers of cell injury with the intention in further part of life en route to progressive complications in T2DM.

P126

Are Uric Acid Values Surrogate for Insulin Resistance in Apparently Healthy Subjects Across a Spectrum of Body Mass Index?

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The concept of insulin resistance initially proposed in diabetic patients is now known to be associated with major public health problems, including obesity, hypertension, coronary artery disease and metabolic syndrome. Serum uric acid values are also elevated in the above conditions and proposed to reflect the insulin-resistant state. Aim was to determine whether serum uric acid levels can be used as a surrogate for Insulin resistance calculated as HOMA-IR in apparently healthy, normal weight, overweight and obese population. Cross sectional study done in 150 subjects of both genders aged 20- 40 years divided equally based on their BMI into 3 group namely normal weight, overweight and obese as per NIH classification. Fasting plasma glucose, fasting serum insulin and serum uric acid were estimated. HOMA-IR was calculated. Mean waist circumference, waist-hip ratio, fasting insulin, fasting glucose, uric acid and HOMA-IR were found to be elevated in both overweight and obese groups. Mean uric acid levels were 4.9, 5.4 and 6.3 and mean HOMA-IR values are 2.2, 3.3 and 7.3 respectively in normal weight, overweight and obese subjects. Significant correlation of uric acid with insulin resistance calculated as HOMA-IR was not found in any of the three groups. There was incremental increase in fasting glucose, fasting insulin, uric acid and HOMA-IR from normal weight to overweight to obese subjects in a systematic proportion. Significant correlation of uric acid with fasting insulin and insulin resistance was not seen and hence cannot be used as the surrogate marker for insulin resistance in apparently healthy population.

P127

Serum Amylase and Lipase Estimation in Diabetic Ketoacidosis (DKA)

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Diabetic ketoacidosis is an acute metabolic complication mainly in type 1 diabetes mellitus(DM). Acute pancreatitis can be a precipitating factor for DKA in patients with diabetes. DKA may mask coexisting acute pancreatitis, which occurs in at least 10-15% cases. Objective was to compare serum amylase and slipase in patients of DKA with DM patients without DKA and in normal healthy controls. Cross sectional study was conducted on 35 known cases of DM with multiple episodes of DKA (Group I), 35 patients with type 1 DM without DKA (Group II) & 35 normal individual as control (Group III). Serum amylase (direct substrate method) and S.lipase (kinetic chromogenic substrate method) were analyzed on fully autoanalyzer at SSG Hospital & Medical College Baroda. Mean of Serum amylase, lipase and random plasma glucose for Group I is149IU/L, 95 IU/L, 461 mg/dl; Group II is 118IU/L, 91IU/L, 318 mg/dl and Group III is 52IU/L, 41IU/L, 104 mg/dl respectively. Normal value of amylase is <90 IU/L & lipase is<60 IU/L. Serum amylase and lipase were significantly high in group I as compared to group III which is statistically significant (P<0.0001). Significant elevations of S. amylase & S. lipase, are more specific for pancreatitis may also accompany DKA.

P128

Assessment of Vitamin D Levels In Type 2 Diabetes Mellitus Patients

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Studies have shown that vitamin D may have a role in pathophysiology of type 2 diabetes mellitus through extra-skeletal vitamin D receptors and its supplementation may influence long term diabetes mellitus control. The aim of our current study was to determine prevalence of vitamin D deficiency in type 2 diabetes mellitus.This was a cross-sectional study done in the Department of Biochemistry, PGIMS, Rohtak. Thirty patients diagnosed with type 2 diabetes mellitus were randomly enrolled in the study after taking informed consent. Patients were divided in to three groups according to measured vitamin D levels, level =20 ng/mL (vitamin D deficient), 21-29 ng/mL (vitamin D insufficient) and =30 ng/mL (vitamin D sufficient). Vitamin D in serum was analysed by enzyme-linked immunosorbent assay. Majority (66.7%) of the diabetic patients were found to be vitamin D deficient. 83.3% female diabetic patients compared to 41.7% male diabetic patients had vitamin D deficiency while 58.3% male patients and 16.7% of female patients had vitamin D insufficiency. The difference was statistically significant (chi-sq. =5.625, P<0.05). Our study showed that majority of type 2 diabetes mellitus patients were deficient in vitamin D levels. Also, there was a considerable difference in male and female values. Thus estimation of vitamin D may help in monitoring of diabetic patients.

P129

Evaluation of Relationship between Gamma Glutamyl Transpeptidase (GGT) and Diagnosed Cases of Type 2 Diabetes Mellitus: A Cross Sectional Study

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Gamma glutamyl transpeptidase (GGT) is a membrane bound enzyme which transfers glutamyl groups linked through the gamma carboxylic acid from peptides such as glutathione to acceptors. Its main physiological function is to make cysteine available for regeneration of intracellular glutathione and thereby protect against oxidative stress. Previous studies showed that high circulating concentration of liver enzymes like GGT, ALT, and AST are increased in individuals with insulin resistance and metabolic syndrome, while GGT might be a stronger risk factor. Objective was to estimate and compare the level of GGT in Type 2 DM patients with that in age and sex matched controls. 30 patients of Type 2 DM in the age group of 40-60 years, attending OPD of GMCH and 30 age and sex matched healthy controls were included in the study. Persons with history of alcoholism, smoking, cardiovascular and renal complications, infectious & hepatocellular diseases and taking any other medications were excluded. In all the subjects,fasting blood glucose, HbA1C and GGT were analyzed by photometry using VITROS 5600 fully autoanalyser and compared statistically. The result showed that GGT levels in diagnosed cases of Type 2 DM was increased significantly (P<0.05) compared to normal controls. In the study, we found a statistically increased level of GGT in Type 2 DM patients. Hence, GGT can be used as a cost effective oxidative stress marker in Type 2 DM.

P130

Study on Levels of Glycosylated Haemoglobin and Urinary Microalbumin in Diabetic Cases and Healthy Controls

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Diabetic nephropathy is a consequence of long standing diabetes. The prevalence of microalbuminuria predicts progression to diabetic nephropathy. The present study was conducted to determine the prevalence of microalbuminuria in relation to HbA_{1C} in Diabetic cases in comparison to healthy controls. This case-control descriptive study was carried out in a Silchar Medical College and Hospital from July 2013 to July 2014. One hundred known diabetic patients with age 21–90 years were included in the study. Informed consent and a structured questionnaire of each patient were recorded. Fasting venous blood and morning urine sample was collected for analysis of Fasting blood glucose, HbA_{1C} and urinary microalbumin respectively. Statistical analysis was done using graph stat statistical software. All P-values <0.05 were considered as statistically significant. Fasting blood glucose levels**,** urinary microalbumin, and blood HbA1C levels were very high in diabetic cases as compared to healthy controls. Also the urinary microalbumin levels were very high in the diabetic cases with poor glycaemic control. The present study found higher level of HbA1C and urinary microalbumin level in diabetics. Also high level of microalbuminuria in the cases which could be due to poor glycaemic control (high HbA1C >7%). Screening for microalbuminuria and HbA1c test should be done in both newly and already diagnosed diabetic patients as an early marker of renal dysfunction and glycaemic control.

P131

Association of Metabolic Syndrome and it's Components with Hypothyroidism

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The metabolic syndrome (MS) and thyroid hypo-function are associated with cardiovascular diseases. The present study was designed (1) to find out occurrence of MS in hypothyroid patients and (2) to determine the association between the components of MS and thyroid hypo-function. One hundred eighteen subjects with the diagnosis of hypothyroidism based on their clinical and thyroid function test profile were included in this cross sectional hospital based descriptive study with their informed consent. The diagnosis of MS was made based on National Cholesterol Education Program (NCEP) Adult Treatment panel (ATP III) criteria. The prevalence of MS was comparable in overt hypothyroidism {56% (n=34)} and subclinical hypothyroidism {60 % (n=35)}. TSH levels were high in metabolic syndrome (14.6±5.42 mIU/L) in comparison to non metabolic syndrome group (11.5±4.8 mIU/L). Overt hypothyroid group had significantly higher serum triglyceride levels (198.1±58.6 mg/dl) than subclinical hypothyroid group (155.29±45.65 mg/dl) and subclinical hypothyroid group had significantly higher fasting plasma glucose (136±33.1 mg/dl) as compared to overt hypothyroid group (111.8±19.2 mg/dl). TSH had significant positive correlation with fasting glucose (r =0.337, P=0.009), diastolic blood pressure (r=0.208, P=0.049) and triglycerides level (r=0.231, P=0.04) in overt hypothyroid cases. Serum fT_3 had significant negative correlation with abdominal obesity in both overt and subclinical hypothyroidism. Screening for the MS in thyroid hypo-function is warranted as it is prevalent in hypothyroidism. Patients with higher TSH in subclinical hypothyroidism are at more risk of developing metabolic syndrome. Thyroid hypo-function, obesity and MS are associated by an intricate mechanism.

P132

Study of Serum Magnesium Levels in Type 2 Diabetes Mellitus Patients

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Diabetes Mellitus is a major public health concern both in developing and developed nations because of its rising prevalence and disabling life threatening complications. Several studies have shown that low serum magnesium levels exists in type 2 diabetes mellitus compared to healthy controls. Magnesium depletion has a negative impact on glucose metabolism and insulin sensitivity in type 2 diabetes mellitus patient with development of complicationslike retinopathy. Objective was to compare the levels of serum magnesium in patients with type 2 diabetes mellitus with that of healthy age and sex matched controls. 30 patients with type 2 diabetes mellitus were taken as cases and 30 age and sex matched individual taken as control. In both the case and the control group, we have measured FBS, PPBS, lipid profile, Urea, Creatinine and magnesium.The result of the study showed significant decrease in the level of magnesium in patients with type 2 diabetes than the healthy control (P<0.05). So it may be concluded that hypomagnesemia exists in patients with type 2 diabetes mellitus and periodic monitoring of serum magnesium levels in patients with type 2 diabetes mellitus may prove to be prudent in clinical practice.

P133

Dosage Related Disparity in Lipid Profile in Omega 3 PUFA Treated Diabetic Rats

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Nutritional supplementation with omega 3 polyunsaturated fatty acids (PUFA) provides an attractive approach that could work additively with established therapies for dyslipidemia in diabetics. Proven beneficial effect of PUFA on lowering of serum TG has been banked on making its consumption unregulated. Effect of high intake of omega 3 on lipid profile in diabetics is less documented, which is presented here. Aim was to determine the effect of omega 3 PUFA on lipid profile in diabetic rats at different doses. Male Wistar rats of 2-3 months old were divided into controls, diabetic control (DC), fish oil low dose (FOLD) and fish oil high dose (FOHD) treated diabetic rats (n=8 in each group). Diabetes was induced by injection of STZ (48 mg/kg, ip). Animals were treated orally for 30 days with a dose of 0.5g/kg/day as low dose and 1g / kg/day as high dose. Analysis of data was done using SPSS version 17. Serum TG and HDL values were significantly (P<0.05) changed within the groups whereas total cholesterol remained non significant among the groups. Post hoc test revealed a significant (P<0.05) increase in TG level in FOHD (166.04±5.61) rats as compared to DC (124.5±14.8). TG level was decreased in FOLD (97.38±13.51). HDL level was significantly decreased in FOHD {8.4(5.2, 8.8)} as compared to FOLD {36.11(17.28, 44.5)}. FOLD had beneficial effect on HDL, increasing it significantly (P<0.05) as compared to the DC {9.7(5.7, 9.7)}. High dose of omega 3 PUFA has an adverse effect on the lipid profile in diabetics. Thus necessitating their judicious consumption.

P134

Association of Metabolic Syndrome with Hypothyroidism: A Study on Meerut Population

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Thyroid functions affect metabolic syndrome parameters including HDL-C, triglycerides, blood pressure and plasma glucose. Thyroid hormone appears to serve as a general pacemaker accelerating metabolic processes and may be associated with Metabolic Syndromes. Objective of the present study was to find out an association between thyroid profile and metabolic syndrome in Meerut population.The present study was conducted on patients with symptoms of hypothyroidismin the Department of Biochemistry, Subharti Medical College, Meerut. Waist circumference, blood pressure, thyroid profile, TG, HDL-C and fasting blood glucose were estimated in all one hundred patients and findings recorded. The data so collected was analyzed using SPSS-16 software package. We found that 57% of hypothyroid patient were suffering from metabolic syndrome, 12% having overt and 45% were suffering from subclinical hypothyroidism and so there is a definite association of metabolic syndrome with hypothyroidism in Meerut population. The prevalence of metabolic syndrome was observed in both overt and subclinical hypothyroid cases. Hence this warrants the intensive screening of both overt and subclinical hypothyroid group.

P135

Identification of Abnormal Hemoglobinopathies during Diabetes Monitoring – Case Reports

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Asymptomatic hemoglobinopathies are common in India which could be associated with monotonous metabolic disorder, diabetes mellitus. We report two known cases of diabetes mellitus, analyzed for HbA1c levels has unusual peaks caused diagnostic dilemma, which were further tested and diagnosed hemoglobinopathies. HbA1c values estimated by HPLC are unreliable in diabetics associated with hemoglobinopathies. Blood samples were collected in BD EDTA vacutainers were used for HbA1c in Bio Rad D10, for Hb electrophoresis in Bio Rad variant II, for CBC in Beckman Coulter and peripheral smear. Serum glucose was estimated on Beckman DxI800 with hexokinase method. In first case, 75 years Punjabi female blood sample analyzed on Bio Rad D10 system to estimate glycocylated hemoglobin showed a small peak at the position of HbA1c comprising 3.7% of total hemoglobin with 88.8% variant window. These values were not matching with her fasting serum glucose levels 192 mg/dL. The other observations on variant II, a large peak on D-window area was 91.5 %, based on these findings we concluded patient has Hemoglobin D homozygous. In second case, 60 years female sample has a large peak of HbA_{1C} 11.8% of total hemoglobin and LA1c/CHb-1 peak 23.7 %**,** which not correlating with her serum glucose levels 183 mg/dL. Findings on Bio Rad variant II, HbA_2: 1.8 %, HbF: 24.6%, suggestive of homozygous HPFH. There are at least two ways in which abnormal hemoglobin may affect HbA1c values; one is the presence of an abnormal peak on HPLC, Secondly, some abnormal forms of hemoglobin make red blood cells more susceptible to hemolysis.

P136

Microalbuminuria and C-Reactive Protein as a Predictor of Coronary Artery Disease in Patients of Acute Chest Pain

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Chest pain is one of the most common challenges for the clinicians in emergency departments. Management strategies are increasingly focusing on preventive measures to reduce the burden of cardiovascular diseases following early detection of markers of atherosclerosis. Biomarkers have become increasingly important in cardiovascular research and disease management. Microalbuminuria and CRP are important putative markers for coronary artery disease in patients of acute chest pain. The present study comprised of 50 patients of acute chest pain of cardiovascular diseases (Group B), 50 patients of acute chest pain secondary to cardiovascular diseases (Group C) and 50 age and sex matched healthy volunteers (Group A). A diagnosis of cardiovascular disease was made from relevant positive history, ECG and related laboratory cardiac biomarkers. 5 ml of venous blood sample was collected from each patient within four hours of admission in emergency wards and CRP was measured. Similarly, early morning midstream urine was collected for micro albumin analysis. Total cholesterol score was calculated and CK-MB activity was also measured in all participants. The usefulness of microalbuminuria, CRP and total cholesterol score was also evaluated in terms of sensitivity, specificity, and positive predictive value. Urinary albumin was calculated to be 12.40±12.81 mg/L in Group A which increased significantly in Group B (30.78±11.33) and Group C (21.88±9.65) respectively. Similarly, CRP was found to be much higher in Group B (almost 6 times with a mean value of 33.82±13.55) and Group C with a mean of 29.72±20.18 in comparison to healthy controls

(5.76±3.91). Sensitivity, specificity and diagnostic accuracy of micro albumin was 82%, 92% and 87%; CRP was 98%, 96% and 97% and for total cholesterol score was 76%, 90% and 83% respectively. Quantification of serum CRP and micro albumin in urine can be used as an important putative biomarker for screening of CVD and may be of significance to clinicians in further management and treatment of such patients.

P137

Evaluation of Antidiabetic and Hypolipidemic Efficacy of Different Fractions of Heartwood of *Pterocarpus marsupium* (Fabaceae) on Alloxan Induced Diabetic Rats

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Antidiabetic plants have the ability to restore the function of damaged pancreatic tissue.The present study was more meaningful because of added advantage of theseplant extracts in being equally effective but without the untoward side effects.The aim of the present study is to evaluate the antidiabetic and antihyperlipidemic effect of different extracts of pterocarpus marsupium and their fractions through oral route of administration in alloxan induced diabetic rats. Diabetes was induced by injecting alloxan. The normal control group was given the vehicle (propylene glycol). The other treatment schedule groups included in the study were insulin treated (6 units/kg body weight). Diabetic control (vehicle only), aqueous treated, Ethyl ether, Petroluem ether, Diethyl ether and Ethyl acetate. All the groups were treated with 75 mg of the extract/kg body weight. Various extracts were dissolved in propylene glycol. After 7 days of stabilisation period alloxan injected group which had more than 200 mg% of blood glucose were included in the study and taken as day 1 for further course of treatment. Blood glucose and lipid profile was also estimated on day 15 and day 30. In the present study there was a highly statistical significance in EA, Aqueous and PE treated groups when compared to DC. There was significant decrease in cholesterol level for DE and PE treated groups when compared to DC and no significant change in triglyceride and HDL levels. The present study suggested the potential of *Pterocarpus marsupium* in diabetes as well as related cardiovascular complications due to its antidiabetic and antihyperlipidemic properties.

P138

Blood lipid levels and Cardiovascular Disease Risk in Obesity

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Obesity is a worldwide health problem, defined as a body mass index (BMI) >29.9 kg/m^2, which is associated with several diseases like type 2 diabetes mellitus, coronary heart disease (CHD), hypertension and certain type of cancer. Obesity is a multifactorial disorder. Among the factors, commonest are social, behavioural, environmental, metabolic and genetic and their interaction with each other regulates the body weight. Imbalance in either of the factors may be responsible for weight gain. Aim of the present study was to evaluate the blood lipid levels and cardiovascular disease risk in obese subjects. This study is conducting in SMS&R, Sharda University, Greater Noida, Santosh Medical College & Hospital, Ghaziabad and IHBAS, New Delhi. Institutional Ethics Committee gave us the permission to conduct this study and after obtaining inform consent, 5ml of blood were collected from each subjects. Diagnosis of obesity is done by modified WHO BMI >29.9 kg/m^2 or WHR >0.9 for men >0.85 for women. Results will be discussed during the presentation as the study is still ongoing.

P139

Effect of *T. Cordifolia* on Lipoprotein Profile in Type 2 Diabetic Patients as Well as Healthy Human Volunteers

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Tinospora cordifolia (Menispermaceae) is widely used in Ayurvedic medicine as a remedy for metabolic disorders however; anti-diabetic and anti-dyslipoproteinemic activities are not well studied in diabetes. This study is carried out to explore these effects in Type 2 Diabetic patients. Aim was to explore effect of *T. cordifolia* on lipoprotein profile in type 2 Diabetic patients as well as healthy human volunteers. Clinical trial was conducted on 30 Type 2 Diabetic patients from Diabetes OPD, as well as 30 healthy human volunteers from families of these patients, following all ethical guide lines. All biochemical assays were done by standard kit methods. A marked increase in levels of fasting plasma glucose, lipid profile accompanied with increase in the lipids and apo-protein

levels of serum β lipoproteins (Very Low Density Lipoprotein and Low Density Lipoprotein), as well as a decrease in lipid and protein constituents of α lipoprotein (High Density Lipoprotein) were observed in Type 2 Diabetic patients as compared to healthy controls. However,oral administration of powdered stem of *T. cordifolia* (50 mg/kg body weight,) for 15 days in diabetic patients resulted in a significant decrease in the levels of fasting plasma glucose, total cholesterol, phospholipids and triglyceride. The decrease of lipids and apoprotein levels of β lipoproteins were followed by stimulation of plasma lecithin cholesterol acyltransferase. Lipid and apoprotein level of α lipoprotein (HDL) were also recovered partially on treatment with *T. cordifolia* On the other hand effect on healthy human volunteers were non significant. The results of the present study leads to research and development of a potent anti diabetic antidyslipoproteinemic drug from *Tinospora cordifolia* extract. *Tinospora cordifolia* may be useful in treatment of different types of dyslipoproteinemia.

P140

Metabolic Syndrome: A Growing Concern for Younger Age in Western U.P.

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The prevalence of Metabolic Syndrome (MetS) represents truely the "tip of ice-berg" phenomenon while the larger actual portion of this emerging non-communicable disease (NCD) remainsubmerged in the general public without being aware. Along with growing prevalence of obesity, northern part of India has witnessed an increasing prevalence of Metabolic Syndrome ranging from 9.2% to 40.4% in various studies. More growing concern is the increasing prevalence of MetS in younger age groups. Objective of the present study was to study the prevalence of different components of MetS in different age groups and in genders in Western U.P. This cross-sectional study was carried out in 161subjects = 16 yrs attending a teaching Hospital in Western UP. 76 subjects were diagnosed to have MetS and 85 subjects were randomly selected as control group. Anthropometric parameters were taken which includes Height, Weight, Waist Circumference and Blood Pressure. Blood sample were collected for Fasting Blood Glucose, Serum TG and HDL-C. 28.9% of the patients of Metabolic Syndrome were 15-25 years of age, 32.9% were between 25-35 years of age, 22.4% were between 35-45 years of age and 14.5% of the patient between 45-55 yrs. There was no gender difference in prevalence of the metabolic syndrome. The increasing prevalence of Metabolic Syndrome in younger age group predispose it to increase risk of Diabetes and Cardiovascular Disorder in remainder of life time. Lifestyle modification and conventional (diet) therapy can improve their outcome.

P141

Prevalence of Metabolic Syndrome in Chronic Institutionalized Patients with Schizophrenia

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Metabolic syndrome (MetS), a constellation of central obesity, hypertension, glucose intolerance and dyslipidaemia, is a serious risk factor for cardiovascular diseases and type-2 diabetes mellitus, both of which are highly prevalent in patients with schizophrenia. Our aim was to study the prevalence of MetS in chronic institutionalized patients with schizophrenia.This cross-sectional study was conducted at Department of Psychiatry, S.M.S. Medical College, Jaipur. 60 schizophrenic male inpatients (with at least 6 months of hospital stay at their last admission), diagnosed according to ICD-10 criteria were screened for prevalence of MetS as per the criteria of the International Diabetes Federation (IDF). The sociodemographic data, clinical characteristics, and medication history was recorded in structured proforma after consent. Morning fasting blood samples were collected between 8.30 to 9.30 am from all patients. Psychopathology was assessed by Positive and Negative Syndrome Scale (PANSS) on the same day. Data were collected and statistically analysed. Among the sixty patients included in the study, prevalence of MetS as per IDF was 32 percent. Only Body Mass Index and waist circumference have predictive and diagnostic accuracy for MetS in patients with schizophrenia. Our findings showed a high risk of MetS in chronic institutionalized patients with schizophrenia. This mandates systematic screening as well as elimination of risk factors such as poor lifestyle, obesity and metabolic disturbances in these indoor patients. Further studies on a larger sample need to be done.

P142

Study of Type 2 Diabetes Mellitus in Women with and without Polycystic Ovary Syndrome

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The polycystic ovary syndrome (PCOS) as well as type 2 diabetes mellitus (T2DM) are common medical conditions linked through insulin resistance. The aim of this study was to investigate the levels of Glucose & enzymatic antioxidant SOD, Catalase, TAC & MDA in patients with T2DM & PCOS.50 women with PCOS, 50 women with T2DM & 50 controls, age & BMI matched controls were evaluated in this controlled clinical study.

Antioxidant enzyme SOD, Catalase, TAC & MDA were estimated. Oxidant status was evaluated by determination of plasma MDA concentration. Women with PCOS had increased prevalence of IGT & T2DM. In PCOS women Glucose, MDA & SOD level were significantly higher (P<0.05). Catalase & TAC level were significantly lower (P>0.05). In T2DM women Glucose, TAC & Catalase were significantly higher (P<0.05). MDA and SOD were significantly lower (P>0.05). The result of this study suggests higher prevalence of PCOS in women with T2DM as compared to non – diabetic subjects.

P143

Association of Serum Uric Acid with Serum Total Cholesterol, Triglyceride and Microalbuminuria in Type-2 Diabetes Mellitus

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Hyperuricemia is common among diabetic patients and Uric acid is also proatherogenic. Lifestyle modifications and medications can easily lower serum uric acid. Therefore, clarifying the role of uric acid in the development and progression of diabetic nephropathy as well as atherosclerosis is of clinical significance. Objective of the present study was to find out if any correlation exists between serum uric acid and serum lipid parameters like total cholesterol and triglyceride and also between serum uric acid and microalbuminuria in type -2 diabetes mellitus cases. Forty-five diabetic patients diagnosed clinically as well as by fasting blood sugar and HbA_1C were included in this study and compared with thirty healthy controls. Mean serum uric acid, total cholesterol and triglyceride levels were found to be significantly elevated in the diabetic cases as compared to controls. In case of diabetics, serum uric acid is significantly correlated with HbA_{1C} (P<0.05). A significant correlation was observed between serum uric acid and microalbuminuria (P<0.05). Serum total cholesterol and triglycerides are not significantly correlated with serum uric acid in this study. Though serum uric acid is not correlated with lipid parameters, the proatherogenic properties of uric acid points towards possible correlation with the early pre-clinical markers of atherosclerosis like carotid intima-media thickness or ankle-brachial index evaluated by ultrasound. Lowering serum uric acid concentration may possibly be effective to lower the degree of urinary albumin excretion in diabetic patients.

P144

Impairment in Glycemic Control and Lipid Profile During Indian Festival Diwali in Type- 2 DM Patients

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Hindu Culture has so many festivals. During festivals there is a tradition of increased consumption of sweets and oil rich fried dishes. One such festival is Diwali celebrated with great zeal and enthusiasm. This likely has an influence on metabolic control of Diabetic patients. The study was conducted on 100 Type- 2 DM Patients (M:F Ratio 60:40: Age 61.82±8.46 years), and 25 healthy control (M:F Ratio 60:40: Age 37.45±5.40 years). Type- 2 DM was diagnosed as per ADA criteria and all the patients were on oral anti-diabetic drugs. Blood samples were collected 2 weeks before and after the Diwali. Patients were instructed to continue with the same oral anti-diabetic medication during this period. Following parameters were measured from the samples: Fasting and post-prandial plasma glucose, Lipid profile (serum total cholesterol, triglycerides, HDL, LDL and VLDL cholesterol). Out of total 100 patients, 74 patients returned for follow-up. There was a significant increase in fasting (136.85±2043 vs.168.62±21.16, P<0.001), and post-prandial (185.22±30.66 vs 217.05±31.16, P<0.001) plasma glucose, total cholesterol (198.47±18.84 vs 212.98±19.06, P<0.001), triglycerides (136.37±25.76 vs 159.44±23.95, P<0.001), LDL (130.25±16.42 vs 142.28±17.50, P<0.001) and VLDL (27.27±5.15 vs 31.68±4.79, P<0.001) cholesterol after Diwali. Whereas, HDL was found to be reduced significantly after Diwali (40.94±3.75 vs 40.01±2.78, P<0.01). Type -2 DM is associated with a cluster of inter related plasma glucose and lipo protein abnormalities. Diwali festival is associated with significant impairment in glycemic control and lipid profile. Therefore around the festive season dietary prevention is always better and prescription must be adjusted in accordance with the change in food intake and patients must be provided with specialized diet counseling to reduce the burden of Type-2 DM complications.

P145

Effects of Opium on Bio-chemical Parameters in Addicts with Non-insulin Dependent Diabetes Mellitus (NIDDM)

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Diabetes mellitus is a chronic metabolic disorder that effects on all parts of the body. The study was designed to determine the effect of opium consumption on biochemical parameters in opium addict persons with non-insulin dependent diabetes mellitus (NIDDM). 25 men 40 -60 years old, with Non- insulin dependent diabetes mellitus addicted to opium as case group and 25 men that suffered from NIDDM non opium addicted as control were selected. The biochemical parameters analyzed includes fasting blood glucose, HbA1c, total cholesterol, triglycerides, HDL, VLDL and LDL – cholesterol, sodium, potassium, calcium, AST, ALT, total proteins, albumin and A:G ratio. Results of both the groups were compared. In diabetic addicts serum glucose, HbA1c, AST and potassium levels were higher ($P<0.01$) and serum ALT, HDL-Cholesterol, serum total protein, albumin and A:G ratio were lower ($P<0.01$) compared to NIDDM non addicts. Consumption of opium and its derivatives alters secretion of regulatory hormones (epinephrine, ACTH, cortisol and glucagon) that increases serum glucose level and decreases HDL-cholesterol level that deteriorates the metabolic regulation in addicts and increases the chances of complications as shown by increase in HbA1c. Further increase in serum potassium level interferes with water regulations that worsen the glycemic control and blood pressure in diabetics. Therefore, opium consumption should be strictly restricted in non insulin dependent diabetes mellitus.

P146

C-Reactive Protein (CRP) and Serum Lipids as an Indicator of Complication in Type-2 Diabetes Mellitus

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Type-II diabetes mellitus is a heterogeneous disorder characterized by beta cell dysfunction and decreased insulin sensitivity. C- reactive protein (CRP) and serum lipids are elevated significantly in uncontrolled diabetic population and also correlate with the severity of diabetes along with presence of various complications. This study was planned to find the correlation of inflammatory biomarkers CRP and serum lipids as an indicator of complication in type-2 diabetes mellitus.This prospective cross sectional study was conducted in the Department of Medicine, SMI Hospital and SGRRIM&HS, Patel Nagar, Dehradun. A total of 52 patients with type-2 diabetes mellitus were selected for the study. The complications were identified by the consultant and the inflammatory biomarkers CRP and lipid profiles were investigated on the first visit. The data were compared using suitable statistical methods. All the subjects had mostly abnormal CRP with a mean of 13.18±8.90 mg/L. CRP levels showed significant positive correlation with FBS, PPS and glycosylated hemoglobin. Dyslipidemia was the commonest complication 32.6% (n=14). Patients with cardiac and retinal complications had significantly higher levels of CRP. The presence of diabetic complication was associated with significantly higher CRP (7.65±1.89 vs. 12.29 ± 3.61, $P<0.05$) by t-test. CRP levels showed significant positive correlation with serum total cholesterol, serum triglyceride and were associated with various diabetic complications. The various complications of type 2 diabetes mellitus had a positive correlation with CRP and lipid profiles, especially serum triglycerides and VLDL levels. This study exhibited an association of diabetic dyslipidemia and chronic inflammation with the pathogenesis of diabetic complications.

P147

Profile of Arthropathies in Type 2 Diabetes Mellitus

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The prevalence of type-2 diabetes has been rapidly rising worldwide. It is a metabolic disease with inappropriate hyperglycemia either due to deficiency of insulin secretion or reduction in the biologic effectiveness of insulin. Arthritis and diseases of the joints have been plaguing mankind since ancient times. There is now a large body of epidemiological evidence linking rheumatoid arthritis (RA) with the premature development of cardiovascular disease and diabetes mellitus. This study was planned to evaluate the relation between rheumatoid arthritis, gout and the future risk of type-2 diabetes. This study includes 80 diabetic patients (32 males; 48 females) and 50 healthy volunteers (16 males; 34 females). The age of diabetic patients ranged from 36 to 70 years and 32 to 55 years for healthy controls. Fasting blood sugar, C-reactive proteins (CRP), RA- factor and uric acid (UA)

concentration were estimated by VITROS 6S auto analyzers. It was found that data is not normally distributed so that log transformation was used to transform the data and check the statistical significance of the data. The present, study includes 130 cases including patients and control group for the correlation of diabetes with various arthropathies. We have done the chi-square test to check the proportion of gender (male and female) in test and control group. To assess the relationship among the variables, Pearson correlation coefficient was estimated. It was found that there is no correlation between FBS and UA in gender group (since P=0.66 and r= NS) which is not significant. On analyzing the data, it was observed that there is a positive correlation between FBS and CRP (r = 0.512;P= 0.0001) in patients. Similar relationship was anticipated between FBS and UA (r=0.393; P = 0.004). However, in control group only UA and CRP shows positive correlation (r=0.32;P=0.023). There is no correlation between FBS and UA but there is only correlation between UA and CRP. There are many meta-analysis reports which showed the same relation as observed in present study, however, the relationship between UA levels and risk of type-2 diabetes showed that each 1 mg/dl increase in UA will result in a 17% increase in the risk of type-2 diabetes. These findings suggest that there are both non causal and causal associations between UA level and the risk of type-2 diabetes. There was no significant difference in the serum uric acid levels between the diabetics and the non-diabetics, either in males or females. Uric acid levels were significantly reduced in insulin-dependent diabetics and in those on oral hypoglycaemics and also in 'non-diabetics' with casual glucose levels >10mmol/l. Further research should attempt to investigate whether UA would be useful for predicting type-2 diabetes and could be of significance in prevention and treatment of the disease.

P148

Serum Vitamin D, Calcium and Magnesium and Glycosylated Haemoglobin in Patients with Type 2 Diabetes Mellitus

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Type II diabetes is prevalent all over the world with an ever increasing number day by day. Consistent deficiency of Vitamin D, Calcium and Magnesium holds a definite basis for the increase of risk for developing the disease in new ones and early onset of diabetic complications in those who already have this disease. The aim of the study was to correlate the serum levels of vitamin D, calcium and magnesium with the levels of glycosylated haemoglobin in diabetic patients of Uttarakhand. It was a hospital based cross sectional study in Dehradun city of Northern India. Thirty six type II diabetic cases were included in the study with age 20 and above of both sexes and compared with thirty six age and sex matched healthy controls. Besides the diagnostic parameters for diabetic patients, other parameters like $25(OH)D_3$, calcium and magnesium were measured in both the groups. The level of $25(OH)D_3$ was found to be decreased in FBS range from 126-200 mg/dl and 200-250 mg/dl with a mean of 13.76±4.06 to 9.75±2.48 respectively. Likewise, with PPBS range from 200- 250 mg/dl to 250-300 mg/dl, serum vitamin $25(OH)D_3$ showed a marked decrease from 12.66±2.22 to 9.27±1.98 respectively. Calcium with FBS range between 126-200 mg/dl and 200-250 mg/dl showed a p-value <0.05 with calcium value 9.14±0.52 *vs* 9.86±0.58 respectively. It showed an increasing trend with increasing PPBS above 200mg/dl, HbA_1C above 8.1% and $25(OH)D_3$ above 10ng/dl. Unlike calcium, opposite results were obtained for magnesium. Magnesium levels were found to decrease with increasing levels of FBS, PPBS and HbA_1C but it did not change significantly with the changing levels of $25(OH)D_3$ in both diabetics and non-diabetics. $25(OH)D_3$ and magnesium levels were significantly lower in diabetics as compared to non diabetics whereas serum Calcium levels were significantly higher in diabetics as compared to non diabetics. There was a inverse correlation between $25(OH)D_3$ levels and serum calcium levels in diabetics but no correlation was found between $25(OH)D_3$ levels and serum magnesium levels in diabetics. Lower values of $25(OH)D_3$ were observed where the number of complications were three or more than three. No correlation was found between the number of complications and serum calcium and magnesium levels.

P149

Circadian Time Structure of Serum 25 (OH) D_3, Calcium and Phosphorus in Type 2 Diabetes Mellitus

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Circadian periodicity, an outgrowth of chronobiology, the study of diversity in time, is the inferential statistical mapping of structures in variables; in and around us, consisting of rhythms, chaos and trends. Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The circadian time structure of serum 25 (OH) D_3 and calcium may relate to prevention and chronotherapeutic efficacy and management. To our knowledge, the diurnal variation of serum 25(OH) D_3 has not yet been reported in type 2 diabetic patients. The present study was planned to evaluate the diurnal variation of serum 25 (OH) D_3, calcium and

phosphorus levels type 2 diabetic patients. Ten clinically healthy volunteers and 10 newly diagnosed patients of type 2 diabetic mellitus of similar age groups were synchronized for one week with diurnal activity from about 06:00 to about 22:00 and nocturnal rest. All subjects took their (although not identical) meals three times daily; breakfast around 08:30, lunch around 13:30 and dinner around 20:30 without any change in their usual fluid intake. They were not taking any drug/neutraceutical that affects studied biochemical profiles, their rhythm and concentration. 5 ml blood samples were collected into plain and sterile vials, under quality control procedures from each participant at 6 hourly intervals at fixed time points for one complete 24-hour span i.e. at 06:00, 12:00, 18:00 and 00:00. Serum were separated from the clotted blood and refrigerated at - 20^{o}C until analyzed the next day. Serum 25(OH) D_3, serum Ca^{++}, serum PO_4, fasting and postprandial blood sugar levels were estimated. A marked diurnal variation in serum 25 (OH) D_3 was recorded in healthy subjects (P=0.030). Similarly, a circadian rhythm of borderline statistical significance was also recorded for vitamin D in diabetic patients (P=0.083) and in healthy participants for serum calcium (P=0.070), phosphorus (P=0.102), and the calcium phosphorus ratio (P=0.091) by the population-mean cosinor analysis. In addition, the amplitude and acrophase differed from healthy participants in diabetic patients for studied variables with a change of MESOR for calcium phosphorus ratio. Mapping the broader time structure of different physiological variables investigated herein may be helpful in understanding the treatment and prevention of diabetic mellitus.

P150

Thyroid Dysfunction in Type-2 Diabetes Mellitus

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The aim of study was to find prevalence of Thyroid dysfunction in patients with type 2 DM, attending OPD and medical Wards in Rama Medical College Hospital & Research Centre, Kanpur. The aim of this study was to evaluate the prevalence of thyroid dysfunction in T-2 Diabetes mellitus. The objective of ongoing study will be thyroid dysfunction in patients suffering from T2 diabetes mellitus against controls. 25 diabetic & 25 non diabetic patients were included who attended OPD and IPD of RMCH&RC Kanpur. These subjects were investigated for total T3, total T4, thyroid stimulating hormone TSH, fasting blood sugar, glycosylated hemoglobin HbA1c, and post parandial blood sugar. The level of T3 &T4 were significantly lower,the level of TSH were higher in T2 diabetics as compared to controls. The prevalence of thyroid dysfunction in T2 diabetes patients is very high, with subclinical hypothyroidism is being most common.

P151

Emergency Tests Requests at Tertiary Cancer Care Laboratory

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One of the most crucial requirements for a Hospital based emergency service is an efficient laboratory back up. The laboratory must provide reliable, efficient, timely services to the emergency patient care. A major problem experienced in many hospitals, centres around, the inappropriate ordering of tests. Objective was to identify the test parameters most frequently requested during the emergency hours, to review the turnaround time (TAT) of the emergency laboratory services and to reduce the inappropriate use of laboratory services during emergency hours. A retrospective analysis of all the test requests received for the month of May-June 2013 during the emergency hours was carried out. Areas most prone to inappropriate use of the services were detected. Following intervention by discussing with the clinicians and reviewing of test which are really emergency, a prospective analysis was done for the requests received for the next six months July-December 2013. A total of 1532 (Biochemistry), 1169 (Haematology) samples were studied between May-December 2013. Following the intervention, decrease in the requests observed was 19.33% (Biochemistry) & 17.61% Haematology. Turnaround time (TAT) for all the samples was maintained as per laboratory protocol. Our findings correlate with those of other centres around the world regarding inappropriate use of emergency laboratory facilities. Periodic analysis of the requests received, which is also one of the quality indicators, should be carried out.

P152

Establishing Syva Methotrexate Assay on Siemens Dimension Rxl Analyser: Experience in a Tertiary Cancer Care Laboratory

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Methotrexate, abbreviated as MTX, is a anti-metabolite and anti-folate drug. Methotrexate test is used for the measurement of methotrexate in human samples, typically serum or plasma. The measurements thus obtained are used in monitoring levels of methotrexate to ensure appropriate drug therapy. The

degree of methotrexate cytotoxicity is related to the drug's concentration and to the duration of exposure. The use of lecovorin "rescue" permits relatively safe administration of very high doses of methotrexate to achieve maximum antineoplastic activity. Monitoring methotrexate concentrations & rate of decline in serum during high-dose therapy is essential when designing adequate leucovorin rescue dosages. Syva Emit Methotrexate Assay is a homogenous enzyme immunoassay intended for use in the quantitative analysis of methotrexate in human serum or plasma. The goal of this study was to establish the Syva Emit Methotrexate Assay on Siemens Dimension RXL analyser and to verify the related parameters. We established Syva Emit Methotrexate Assay on the Siemens Dimension RxL analyser for Methotrexate evaluation. All the quality parameters like CV%, Precision, Linearity, Limit of detection, limit of blank, inter-lab comparison were assessed for the assay. CV% of 6.4, Linearity of 0.17-1.98 μMol/L, Limit of detection 0.17 μMol/L and Limit of blank 0 μMol/L were observed. Syva Emit Methotrexate Assay can be successfully established on Siemens Dimension RXL analyser and used in the evaluation of methotrexate levels

P153

A Case Study-Leber's Hereditary Optic Neuropathy: An Important Cause of Progressive Painless Visual Loss

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Leber's Hereditary Optic Neuropathy (LHON) is a maternally transmitted disease of the optic nerve that primarily affects males in their second or third decade. Clinical features include painless acute or subacute loss of vision, deep central scotomas, disc edema,hyperemia and peri-papillary telangiectasia. Patients are otherwise healthy. Mitochondria is a generator of ATP, required for the all the functions of the body.The major types of mutations in this disease are:(1)m.3460G>A (guanine to adenosine); (2)m.11778G>A (guanine to adenosine); (3)m.14484T>C (thymidine to cytosine) disrupt key polypeptide subunits of complex I respiratory chain. The final pathological outcome in LHON is apoptotic retinal ganglion cells loss. Objective was to find out the gene mutations in a young male patient with clinical and neurophysiological condition suggestive of LHON, confirmed by genetic testing.A case study was done on 20 year old male patient in neuromedicine OPD in Sri Krishna Hospital, Karamsad after the consent of the patient. Molecular genetic testing for the three common LHON mitochondrial DNA point mutations (targeted mutation analysis) in the patient and his three unaffected sibling sisters were done for confirmation of LHON. Affected male patient on molecular genetic testing revealed mutation on 11778G>A and his three sibling sisters revealed same type of mutation but phenotypically were normal. LHON is a mitochondrial genetic disease characterised by bilateral subacute loss of central vision owing to focal degeneration of the optic nerve. The vast majority of cases are result of one of three mtDNA point mutations.To confirm these mutations molecular genetic testing is now available.

P154

Antibiotic Sensitivity and Phenotypic Detection of ESBL Producing E.coli Strains Causing Urinary Tract Infection in a Community Hospital Chennai, India

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Urinary Tract Infection (UTI) forms the largest single group of hospital acquired infections and account for about 40-50% of the total nosocomial infections. In spite of the wide spread availability of antibiotics, UTI remains to be one of the most common infectious diseases diagnosed. Further world wide data shows that there is an increasing resistance among UTI pathogens to conventional drugs. Resistance has emerged even to newer more potent antimicrobial agents. Therefore, the aim of the present study was to determine the prevalence and susceptibility of extended spectrum beta – lactamase in urinary isolates of *Escherichia coli* (*E. coli*) in a community hospital, Sundaram Medical Foundation, Chennai, South India. A total number of 562 urine samples suspicious of UTI were analyzed and it was found that 115 cultures were positive for *E. coli* infection. The study period ranges from March to April 2012. Antimicrobial susceptibility testing was determined to commonly used antibiotics using the modified Kirby-Bauer's disc diffusion method. ESBL detection was done by the screening method of double disc synergy test and then confirmed by the phenotypic confirmatory test with combination disc as recommended by the Clinical Laboratory Standards Institute (CLSI) and the minimum inhibitory concentration (MIC) method using the E-test strips (AB Biodisk, Sweden). The prevalence of ESBL *E. coli* was 34.8%. The ESBL producing isolates were significantly resistant to Ampicillin (100%), norfloxacin (98%) and Nalidixic acid (100%) and third generation of cephalosporins (100%) as compared to non-ESBL producers. Multidrug resistance was significantly higher (63.2%) in ESBL positive isolates than non-ESBL isolates (26.3%). Knowledge of the prevalence of ESBL and resistance pattern of bacterial isolates in a geographical area will help the clinicians to formulate the guidelines for antibiotic therapy to avoid inappropriate use of extended spectrum cephalosporins. In conclusion ESBL producing *E. coli* can be treated with beta lactamase inhibitors like Augumentin and Tazobactum/Pippercillin to some extent. As carbapenems like Imipenem and Ertapenem sensitivity is high, therefore these drugs are the only choice for the treatment of severe or life threatening infections caused by ESBL

producing organisms. In order to prevent the outbreaks of this life threatening ESBL producers, certain infection control measures have to be followed. Adequate precautions have to be taken to minimize the risk of cross contamination among patients. Contact precautions by cohort patients during outbreaks and also promoting meticulous hand hygiene practices.

P155

Comparison of Immunoassay Kits for Anti-Thyroperoxidase Autoantibody

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Thyroperoxidase (TPO) is a membrane-bound enzyme in the thyroid cell, essential for the thyroid hormone synthesis. It is one of the three thyroid antigens responsible for the development of autoimmune thyroid diseases. At RMC, a large number of samples are received annually, which are being analyzed for anti-TPO by agglutination method. We aimed to replace the existing method with a sensitive radioimmunoassay for the quantitative determination of anti-TPO. Comparison of 3 commercial kits viz. Immunotech (RIA), France; Selco (RIA), Germany and SERODIA-AMC (agglutination), Fujirebio Inc. Tokyo, Japan was done with serum samples of patients suffering from autoimmune thyroid diseases. Samples (n=41) were selected on the basis of varying antibody titres as determined by agglutination method. The samples positive for anti-TPO levels were 19 (n=41), 20 (n=33) and 22 (n=41) using the agglutination kit, Selco RIA kit and Immmunotech kit respectively. Both the RIA methods were sensitive compared to the agglutination kit. On comparison, Selco and Immunotech RIA kits showed a good correlation ($r=0.80$, $n=33$, $P<0.001$). Agglutination method being a semi-quantitative test could not be correlated with the RIA kits. Five samples negative for anti-TPO levels by agglutination method were positive by one or both the RIA methods.Results obtained using both the RIA kits were satisfactory. Hence, either of the RIA kits could be used depending on availability, cost and convenience.

P156

Study of Lipid Profile in Pulmonary Tuberculosis Patients and Relapse Cases in Relation with Disease Severity -A Pilot Study

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Malnutrition and Tuberculosis are synergistically associated to each other. Any inflammatory condition following infection causes release of free radicals and reactive oxygen species (ROS) which adversely affects host lipid by causing enhanced lipid peroxidation. The objective of the study was to determine the level of lipid fractions in newly diagnosed and relapsed Pulmonary Tuberculosis patients and to correlate serum lipid level with inflammation and disease severity. 32 newly diagnosed and 26 relapsed cases to PTB were recruited for the study. Patients were both male and female with average age 37.16±1.2 years and 39.44±1.5 years respectively. 25 age and gender matched healthy subjects that were non family members of patients were taken as controls for comparison. Fasting serum lipid profile Total Cholesterol (TC),Triglyceride (TG), HDL-cholesterol (HDL-C), Low density Lipoprotein (LDL) and Very Low density Lipoprotein (VLDL)) and CRP along with ADA were estimated**.** All lipid parameters were significantly ($P<0.05$) low in both newly diagnosed and relapse cases of Pulmonary Tuberculosis (PTB) than controls. TC and LDL level were significantly higher in relapsed patients than new PTB cases. Inflammatory markers (ADA and CRP) increased significantly ($P<0.05$) in both new and relapsed group according to control group. Cholesterol and LDL are moderately correlated to serum ADA as compared to CRP, however no significant correlation was observed between other lipid parameters with ADA or CRP. However, lipid parameters are well correlated with smear positivity extent (SPE) indicating that SPE is a better measure to assess disease severity which involves progressive decrease in serum lipids**.** Hypocholesterolemia exists in both newly diagnosed and relapse PTB patients and is one of the many nutritional factors predisposing for TB infection. Serum lipids affect overall strength of immune system with cholesterol being most widely studied in this aspect. SPE shows strong correlation with serum lipids in PTB patients, indicating its reliability in assessing dyslipidemia in PTB patients.

P157

Knowledge of Health Care Providers in Tertiary Care Level Hospital in Udaipur Towards HIV

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HIV/ AIDS is pandemic involving globe including India. There are increasing trends of HIV infection since 1986. Infected patients of HIV are a huge burden on government for health care facility. The prevalence of AIDS in India in 2013 was 0.27. Those who are positive for HIV/AIDS the health care workers are the focus for prevention and management of HIV. The knowledge, Practice, Perception & moral of health care workers toward HIV patients is the key for the control of HIV in India. This study is conducted to - know the Attitude and knowledge of the Health care providers in tertiary care level hospital, Udaipur towards HIV and to identify the content of need of training to Health care providers in regard to HIV/ AIDS. A cross sectional study is conducted from June 2014 to July 2014 in Tertiary care level hospital, GMCH, Udaipur. Study subjects selected for the study were 76 comprising of nursing staff selected randomly. The information regarding Knowledge towards HIV transmission & provision of health care was recorded in a pretested proforma. In this study, the majority among the study subjects were males with the age range included 21 – 40 years. The knowledge of the study subjects toward routes of transmission in majority was blood (94.7%) & unsafe sex (92.1%). Majority of the study subjects had some knowledge about the diagnosis. In this study nursing staff among the study subjects showed their interest for the care of HIV positive patients. It is important that 60.5% of the study subjects were in favour of early sex education. Although the study subjects had some knowledge regarding HIV infection but there is a need of training to health care providers for the control & prevention of dreadful disease HIV/ AIDS. For social mobilization regarding various aspects of HIV there is a need of School health education.

P158

A Study on Blood Donation Among Medical Students of Udaipur

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Blood borne disorders specially Hep-B and HIV are the major threat to globe as well as India. Morden inventions on fast transport are other important contributors for increasing trend of accidents. Blood is needed for emergency situation like motor vehicle accidents, blood borne disorders and anaemic condition in India. Blood donation is most important elements for saving the human life and there is no substitute for it. Blood donation should be done judiciously because contaminated blood may transfer many of the blood borne disorders. India needed about 7 million unit blood annually. Aim was to know the knowledge and attitude towards blood donation among medical college students. This is a cross sectional study conducted on first MBBS students of GMCH, Udaipur during august 2013 to October 2013. The study subjects were 123 and information regarding knowledge and attitude for blood donation was recorded in a pre tested proforma, after obtaining the oral and written consent from study subjects. Out of 123 students the girls and boys were same in number. Majority of the study participants 55.2% belonged to 18 years of age. The knowledge regarding universal donor i.e. blood group "O" and "AB" universal recipient is known to 78% and 76.4% of the study subjects respectively. Majority 82.1% of the study subjects had the knowledge regarding 90 days of duration, further blood can be donated if already have donated earlier. 86.1% of the medical students have not donated blood till date and those who have donated 52.0%, their source of knowledge for donation of blood was television.In this study it is evident that the frequency of blood donation by medical students is very low. If medical college students set a example of blood donation on the eve of world donation day that will motivate and boost morale of health care providers for saving the human lives.

P159

Mycophenolate Mofetil Increases Gene Expression of Cell Surface Associated Mucins in Primary Cultures of Oral Mucosal Epithelial Cells

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Autologous cultured explants of human oral mucosal epithelial cells (OMEC) are a potential therapeutic modality for bilateral ocular surface disease (OSD). Most of these patients suffer from incapacitating dry eye with significantly reduced mucin production. Mycophenolate mofetil (MMF) has been shown to upregulate the mucin expression in conjunctival goblet cells *in vitro*. The aim was to evaluate the effects of MMF on mucin gene expression in primary cultures of oral mucosal epithelial cells.With informed consent, oral mucosal epithelial tissue samples were obtained from patients undergoing oral surgery for non-malignant conditions. OMEC were grown on human amniotic membrane scaffold for 2 weeks in growth media containing DMEM & Ham's F12 with FBS and growth factors. Mucin gene expression was quantified using RT-PCR and q-PCR before and after treating cultured OMEC with MMF for 24

hours. Morphological studies revealed a confluent sheet of proliferating, stratified oral mucosal epithelial cells. The presence of cell surface associated mucin genes (MUC1, MUC15 and MUC16) was elucidated by RT-PCR. The expression of mucin genes was found to be upregulated in MMF treated primary cultures of OMEC, compared to untreated controls as quantified by q-PCR with β-actin as internal control. Our findings demonstrate that MMF can be a novel enhancer of mucin production in OMEC *in vitro*. It has the potential to improve dry eye in patients undergoing OMEC transplantation for bilateral OSD. Further clinical trials are required to establish the role of MMF in patients undergoing OMEC transplantation.

P160

Evaluation of Leech Therapy in Psoriatic Arthritis as Compared to Traditional Ayurvedic Therapy

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Medicinal leech *(Hirudo medicinalis)* has been found to secrete saliva containing about 60 different proteins. These achieve a wide variety of goals useful to the leech as it feeds, helping to keep the blood in liquid form and increasing blood flow in the affected area. Several of these secreted proteins serve as anticoagulants, platelet aggregation inhibitors,vasodilators, and proteinase inhibitors. It is also thought that the saliva contains an anesthetic, as leech bites are generally not painful. Leech therapy has been used in ayurvedic medicine for various diseases and one of them is psoriasis. Leech therapy is better than traditional ayurvedic therapy with Khadir ghan satva (sanshaman) as interpreted by estimating the levels of vit C, glutathione, superoxide dismutase, malondialdehyde, TNF alpha, IL-6 in plasma. Study consisted of 60 cases divided into 2 groups, one group of 30 patients receiving only ayurvedic therapy with Khadir ghan satva and another group of 30 patients receiving only leech therapy. Blood was collected from each group before and after treatment. Inflammatory markers were found to be reduced more in patients with leech therapy than the patients receiving traditional ayurvedic therapy ($P<0.001$). Leech therapy is superior to the traditional ayurvedic therapy as shown by the inflammatory and oxidative stress markers.

P161

Cord Blood Insulin Levels: Correlation With Gender, Birth Weight and Placental Weight in Term Newborns

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Fetal development is a complex multifactorial process. The most important hormone in the development of fetus is insulin and along with insulin like growth factoralso influences the development of placenta. Female fetus is less responsive to the trophic effects of insulin and is therefore smaller in size and has lesser weight. The aim was to determine the reference levels of cord blood glucose and insulin in term newborns, correlate insulin levels with gender of the baby and find out the correlation of insulin with birth weight & placental weight. Cross sectional study comprising 60 consecutive term newborns from KMC constituent hospitals. The placental and birth weights were measured by the same weighing machine and cord blood was collected for estimation of serum insulin and plasma glucose. Plasma glucose was estimated by GOD-POD method using auto analyzer and serum insulin analysis by ELISA. In 60 term newborns (23 males and 37 females), mean ± 2SD for cord blood insulin and glucose values were 10.1 ± 7.8μU/mL and 67.8 ± 33.8 mg/dl respectively. There is correlation with both birth weight and placental weight ($r = 0.35$) and ($r = 0.41$) respectively. We found female babies were of lesser birth weight but had higher cord insulin levels than males. Study suggests the reference level for cord blood insulin and glucose in term newborns. Female newborn babies with higher insulin levels than males despite lesser birth weight can be attributed to an intrinsic insulin resistance.

P162

Prostate Specific Antigen in Cord Blood: It's Correlation with Gestational Age, Mode of Delivery, Birth Weight and Gender of Newborns

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Recent literature surveys have detected PSA levels in cord blood of male as well as female babies yet very little has been studied regarding its significance. The placental hormones up-regulate the secretion of PSA in placenta. This leads us to the hypothesis that PSA might have a role in fetal growth and development. The aim

of the study was to determine the concentration of prostate specific antigen in cord blood and correlate it with the gestational age, mode of delivery, birth weight and gender of the newborn. 73 cord blood samples were collected from the umbilical cord during deliveriesin KMC constituent hospitals and analyzed for PSA using 'Calbiotech Inc. PSA ELISA kit'. Mean PSA levels in cord blood was found to be 0.282 ± 0.09 ng/ml. The PSA levels were found to increase with increasing gestational age (mean in preterm was 0.198 ± 0.07 and in term 0.307 ± 0.07 ng/ml; P< 0.001). Mean PSA levels was found to be higher in case of normal vaginal delivery (0.29 ± 0.08 ng/ml) than operative delivery (0.154 ± 0.004 ng/ml) and the statistical correlation with mode of delivery was highly significant (P<0.001). PSA levels increased with increasing birth weight but there was no significant difference between PSA levels of male and female babies. Correlation of PSA with gestational age was highly significant suggesting its role in fetal growth and maturity. This can be attributed to its proteolytic action on substrates like insulin chains, insulin like growth factors etc. Higher values of PSA in cord blood of bigger babies found in this study suggest PSA as an anabolic molecule. The higher stress and strain of vaginal delivery than cesarean section results in increased adrenal glucocorticoids leading to higher PSA levels.

P163

Correlation Between Hormonal Status & Lipid Levels in Perimenopausal & Postmenopausal Women

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The hormonal changes associated with menopause play important role in derangement of serum lipid levels and most cardiac related abnormalities. The aim of the present study was to correlate the serum lipid levels and the influence of estradiol, FSH, & LH on the lipid levels in apparently healthy perimenopausal & postmenopausal women. 25 apparently healthy perimenopausal (45-49 years) & 25 postmenopausal (50-55 years) women were selected for the study. The subjects having risk factors that may affect the lipid levels and hormonal status were excluded. Serum triglyceride, cholesterol and HDL, LDL were measured by standard kit method on fully automated autoanalyser. Serum hormone levels were measured by ELISA kit using chemiluminiscent technology. Student's t test and Pearson's test of correlation were used for the statistical analysis. As compared to the perimenopausal women, postmenopausal women had higher but non-significant (P>0.05) concentrations of total cholesterol, LDL and triglycerides. The concentration of estradiol were significantly (P<0.05) lower in postmenopausal than perimenopausal women. The concentration of FSH, LH were significantly (P<0.05) higher in postmenopausal than perimenopausal women. Estradiol concentration had non-significant (P>0.05) negative correlation with total cholesterol and LDL. The concentrations of FSH, LH had non-significantly (P>0.05) positive correlation with total cholesterol and LDL. Menopause leads to changes in lipid profile by increasing total cholesterol and LDL, thus increasing the risk for cardiovascular disease. These changes are due to decrease in the level of estrogen in menopause.

P164

Myeloperoxidase Activity and Nitric Oxide Levels in Acute Myocardial Infarction

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Oxidative damage plays an important role in the pathogenesis of several human diseases. Myeloperoxidase (MPO) and Nitric oxide (NO) are involved in tissue injury in different disorders. The objective was to study the levels of Malondialdehyde (MDA) and NO and activity of MPO in Acute Myocardial Infarction (AMI). Serum MPO activity, levels of NO and Malondialdehyde (MDA) were determined in 50 AMI patients and 50 normal healthy control. MPO activity was estimated by spectrophotometric method of Weiss and co workers, NO level were estimated by Kinetic cadmium Reduction method, and MDA levels were determined by method of K Satoh. Study showed significantly increased activity of MPO (P<0.0001) and levels of MDA(P<0.006) in AMI patients (101.9±65.3 mU/ml and 5.14±2.2 nmol/ml) compared to controls (34.3±13.6 mU/ml and 3.82±1.7 nmol/ml). The levels of NO were found to be significantly reduced (P<0.0001) in AMI patients (20.48 ± 6.1) in comparison with controls (36.17 ± 7.0). Increased activity of MPO and depletion of NO may induce lipid peroxidation**.** This oxidative stress might contribute to pathogenesis of AMI.

P165

Thyroid Dysfunction in Thalassemia Major

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Thalassemia major is an inherited hemoglobinopathy characterized by chronic anemia and primary hypothyroidism is one of the most frequent complications observed in-patients suffering from thalassemia. We investigated and reviewed the thyroid function in all thalassemic patients attending the Pediatric ward of Grant Govt Medical College. A total of 30 patients were studied between age group of 5 years and 18 years of age. Thyroid hormone estimation was done by chemiluminiscence assay by

IMMULITE 1000. 40% of the patients show thyroid hormone level below normal. The data demonstrates the occurrence of impaired endocrine function in the beta-thalassaemia and it may be due to iron overload.

P166

Correlation of Lipoprotein (a) Levels by Electrophoresis with Nephelometry

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Lipoproteins are water-soluble carrier molecules made of lipids and proteins which are synthesized in the liver and the intestine. Lp(a) is a sub class of lipoproteins which are direct indicators for the prevalence of Coronary Artery Disease. The purpose of this study is to detect Lp(a) by electrophoresis and nephelometry and compare the results of the two methods. 72 blood samples were collected and serum was analysed for Lp(a) by Electrophoresis and Nephelometry.The lipoprotein (a) was detected by electrophoresis on buffered agarose gels on the semi automated SEBIA HYDRASYS instrument and quantitatively measured on the Immage Immunochemistry system by rate nephelometry. Lp(a) band was observed between Alpha and Pre-beta region on electrophoresis. 31 samples without Lp(a) band on electrophoresis showed levels< 30 mg/dl when measured by Nephelometer, whereas 41 samples with Lp(a) band on electrophoresis showed levels > 30 mg/dl when measured by Nephelometer. Strong positive correlation between the two methods was observed with Pearson correlation coefficient of 0.76 (P< 0.0001). We conclude that Lp(a) detection by electrophoresis correlates well with quantitation by nephelometric method. Also, Lp(a) band < 2% indicates that the electrophoretic pattern is within normal limits and correlates with normal reference limits of < 30 mg/dl by nephelometric method.

P167

Evaluation of Metallic Toxicity of the Lead used in *Nagabhasma*- A Lead Based Ayurvedic Medicine

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Even though lead is the major content of the *nagabhasma*, it has been claimed that using the stringent traditional methods metallic toxicity is removed and the medicinal properties are imbibed during its purification. Therefore, the present study is designed to evaluate the level of toxicity of lead in various stages of the preparation of *nagabhasma. Naghabhasma* is an ayurvedic medicine mainly made from the lead. As the lead is highly toxic to the human health, various studies have shown the toxicity of traditional medicines containing lead. Human equivalent dose of various stages of *nagabhasma* (stage I to IV) was administered orally to the healthy Wistar rats for 30 days. At the end of the treatment, blood was collected and analyzed for liver and kidney function test along with estimation of oxidative stress. At the end of the treatment period, the lowest level of blood lead was found in animals fed with stage IV bhasma. Serum ALT, AST, ALP, Creatinine, Urea, GST, MDA and Thiol levels were significantly high in animals fed with stage I bhasma and toxic levels were gradually decreased in animals fed with stage II, III and IV bhasma. The results indicate that the toxic effect of lead in the bhasma on kidney, liver and on oxidative stress is decreasing as the stages of lead processing are advanced. This clearly indicates that, the stringent traditional way of preparation of the bhasma is important to overcome the toxic effect of the metallic lead.

P168

A Study on Lipid Profile in Subclinical Hypothyroidism

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Subclinical hypothyroidism (SCH) is defined as a serum TSH concentration above the statistically defined upper limit of the reference range when serum free T4 (fT4) concentration is within its reference range**.** The decision about whether to screen patients for subclinical hypothyroidism is clouded by inconsistent evidence of dyslipidemia and other risk factors of cardiovascular disease with SCH and also any benefit from early treatment. Our aim was to determine whether the lipid abnormalities are more significant in patients with subclinical hypothyroid patients as compared to control. A total of 60 subjects comprising 30 subclinical hypothyroidism patients and 30 age and sex matched healthy controls were included in the study. In all the subjects fasting blood glucose, and lipid profile tests were analyzed by photometry. We found that total cholesterol, triglycerides were significantly higher in SCH patients than in controls and were statistically significant (P<0.05). The percentage of subjects having higher BMI, elevated TC, LDL, TGL and decreased HDL was higher in SCH patient than in controls. Among the SCH patients, TSH level were positively correlated with elevated triglycerides and total cholesterol. There were higher prevalence rates for undesirable lipid profiles in SCH patients in our study which seem to increase the cardiovascular risk and weight in favor of treatment of patients with subclinical hypothyroidism.

P169

Study of Lipid Profile and C-Reactive Protein in Pre and Post-Menopausal Women

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Menopause is characterized by decrease level of estrogen and large number of hormonal changes. Estrogen has cardioprotective effect in maintaining lipid profile status. C-reactive protein also plays an active role in atherogenesis. Since cardiovascular disease is a leading cause of mortality among post-menopausal women determination of its risk factors bears great importance. Our aim was to evaluate lipid profile status and C-reactive protein level in post-menopausal women and compare with pre-menopausal women. 30 cases (post-menopausal women) were included in the study and 30 regularly menstruating women in the reproductive age group were taken as control. In both the study groups we have measured lipid profile which includes (TC, TG, HDL-C, LDL-C, VLDL-C) and C-reactive protein (CRP) by photometry. Results were analyzed by standard Statistical tests.The results of this study showed significantly increasedlevel of serum total cholesterol, TG, LDL-C and VLDL-C in the cases compared to control group ($P<0.05$), while serum HDL-C level is significantly lower in cases compared to control ($P<0.05$). It also showed elevated mean LDL-C to HDL-C ratio and increased level of CRP in the cases compared to control ($P<0.05$). The results of our study provide information that cardiovascular risk factors are elevated in post-menopausal women compared to pre-menopausal women and addition of CRP to traditional lipid screening enhances the prediction of cardiovascular risk.

P170

Fruit of *Withania coagulans* Extract Ameliorates the Activity of Tyrosine Kinase and GLUT4 and Stimulates the Release of Insulin from Pancreatic β-cell Islets

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Withania coagulans fruit is widely used for its anti-hyperglycemic effect in the traditional health care system in India. The aim of this study was to evaluate insulinotropic effects of aqueous extract of *Withania coagulans* (aqWC) from pancreatic â-cell islets. Diabetic animals were treated with aqWC extract for 30 days. Tyrosine kinase activity was assayed in RBC and hepatocytes and GLUT4 expression in muscle tissue. At the end of treatment, pancreatic islets were isolated, suspended in culture medium with glucose at 3 mM (normoglycemic concentration) and 11 mM (hyperglycemic concentration) and studied the insulinotropic effect of aqWC. Diabetic animals treated with aqWC at a dose of 250 mg/kg showed significantly decreased fasting and postprandial plasma glucose level and increased circulating insulin and C-peptide levels as compared to diabetic-untreated animals ($P<0.001$). Similarly, tyrosine kinase activity in RBCs and hepatocytes and GLUT4 expression were significantly increased in diabetic treated with aqWC for 30 days as compared to diabetic untreated animals ($P<0.01$). Release of insulin from pancreatic β-cells islets isolated from healthy controls and diabetic-untreated animals was studied using two different concentrations of glucose, i.e. 3mM and 11mM in the medium. The release of insulin from islets was increased in 11 mM of glucose as compared to 3mM glucose. Further addition of aqWC to the incubation mixture showed more release of insulin from pancreatic islets ($P<0.05$). However, the release of insulin from islets isolated from healthy animals was higher as compared to diabetic animals. These results suggest that aqWC has effective glucose lowering potential which might be due to increased tyrosine kinase activity as well as the insulinotropic effect leading to increased insulin secretion from pancreatic β cells.

P171

In Vitro Antifungal Activity and Probable Fungicidal Mechanism of Aqueous Extract of *Barleria grandiflora*

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Barleria grandiflora Dalz (Acanthaceae) is being used in India to treat different types of disorders, including skin infections. Therefore, there were good possibilities to have antifungal compounds in its extracts. The objective was evaluation of antifungal activity of plant extract on *A. fumigatus* metabolic pathway.The microbroth dilution assay was used to explore antifungal activity and MIC of various extracts. For discrimination analysis, the metabolomic profiles of control and treated cultures were collected from Agilent 6538 Accurate-Mass Q-TOF interfaced with Agilent 1290 Infinity Series HPLC. Antifungal activities were observed in hot and cold water extracts. Hot water extract of *B. grandiflora* showed significant activity against tested fungi in the range 0.625- 1.25 mg/mL. Partial least discrimination analysis revealed that the active plant extract down-regulated amino acid, glyoxylate pathway and methylcitrate pathways at the same time due to synergy effects of secondary metabolites. Hot water extract showed promising antifungal activity which can be further exploited by identification of active compound. Hot water extract down-regulated several metabolomic pathways unique to fungi indicating its specific activity toward fungi. Extract can be further explored to identify molecules which inhibit specific pathway to develop or design antifungal drugs.

P172

Clinicopathological Study in Celiac Iceberg of North India

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Celiac disease is an autoimmune disorder caused by the ingestion of wheat gluten and related proteins in genetically susceptible individuals. It is characterized by anti-tissue transglutaminase (anti-tTG) antibodies. An intestinal biopsy together with positive serology represents the gold standard in diagnosing celiac disease. Correlation of clinical, serologic, and histological features is essential for a definitive diagnosis.The study aimed to explore clinical presentations, serum tTG IgA levels and small intestinal biopsy results in patients suspected of celiac disease (CD). Sixty patients suspected to have celiac disease were studied. Anti-tTG IgA levels were performed by enzyme linked immune-sorbent assay (ELISA). The cutoff value of 7 U/mL was taken to define positive andnegative results of anti- tTG IgA. Duodenal biopsy in addition to serological assessment was done.The study group included 27 (45%) males and 33 (55%) females withmean age of 20.84 ± 17.92 years (1.5-73 years). Chronic diarrhea, pain abdomen, failure to thrive, anemia, short stature and anorexia were the common modes of presentation.Anti-tTG IgA levels ranged from 0.59 to >1000 U/ml. Increased awareness among clinicians, biochemists and pathologists about CD will aid in diagnosing more cases from the "celiac iceberg". This will relieve a distinct subset of population of distressing symptoms and associated complications by lifestyle modifications.

P173

Global Prevalence of *MTHFR* C677T Gene Polymorphism: A Meta-analysis of Population Based Studies

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Methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism has been reported as a risk factor for several diseases and /or clinical conditions such as- Down syndrome, neural tube defects, cardiovascular diseases, schizophrenia, cancer etc. This SNP has been described from several populations from all over the world.The objective of the study was to estimate the global prevalence of this polymorphism and characterize its regional variation, accounting for differences in population by conducting a meta-analysis. Pubmed, Science direct, Springer link and Google scholar databases were searched for eligible studies. Pooled prevalence proportions (PP) and 95% CIs were estimated on the basis of the individual PPs. Sub-group analysis based on geographical area (i.e. Africa, America, Asia and Europe) was also performed. All P values were two tailed with a significance level at 0.05 and meta-analyses were performed with Meta-analyst program. The frequency of T allele was 35.4 (95% CI: 35.0-35.7) in all studies. In subgroup analysis lowest frequency was found in African (17.6; 95% CI: 15.3-20.1) and highest in Asian (37.2; 95% CI: 36.7-37.6) studies. The frequency of TT genotype was 16.9 (95%CI: 16.5-17.3). In subgroup analysis lowest frequency was found in African (7.2; 95% CI: 4.7-10.9) and highest in Asian (19.2; 95% CI: 18.7-19.7) studies. The present meta-analysis showed that *MTHFR* C677T polymorphism varies greatly in different continents

and/or countries. Populations with higher T allele may be at the risk of developing diseases/disorders associated with this polymorphism.

P174

Biochemical Analysis of Biological Exhibit (venom) Received in Forensic Laboratory- A Case Study

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Forensics is the science in the service of Crime Detection, Law and Justice. Detection, species typing and blood-grouping of biological material using biochemical analysis are major work areas in any Forensic Lab. Crimes against wildlife include illegal hunting, trafficking of endangered species, and producing and selling products made from endangered or threatened species. Our objective was examination of biological material (venom), its characterization and species identification. A biological sample suspected to be venom was seized from poachers. These samples are sold in the market as venoms at exorbitantly high price. All the exhibits were forwarded to Reg. Forensic Science Laboratory, Pune. Cross over electrophoresis was used for determining the species origin of this biological exhibit.Thorough examination revealed; the sample is snake venom which in normal condition was showing negative test with antivenom. But proper processing of the sample using appropriate emulsifier unmasked the venom. Every criminal can be connected to his crime by the fact that the criminal leaves or carries the evidence on or from the crime scene. This is the basis of the crime investigation. Biochemical tests are the tools to link the evidence with crime scene, only if explored in proper scientific manner which don't allow theaccuse to escape even if such crimes are performed using scientific intellect.

P175

Bone Mineral Density and Urine Hydoxyproline Levels Screening in Patients with Epilepsy: before Treatment

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Patients with epilepsy are at increased risk of developing bone abnormalities due to defective mineralisation and antiepileptic drugsassociated bone resorption. There is relatively long lasting depolarisation of neuronal membrane due to influx of extracellular calcium resulting in release of glutamate. In previous studies bone mineral density (BMD) was found to be reduced in patients receiving AED's. Evidences in epilepsy research have shown some genetic mutations in ion channel functions that can affect bone metabolism directly. Hence, we estimated Bone mineral density and urine hydroxyproline that is a marker of bone resorption, in patients with epilepsy at the time of presentation but not on any medication. Study included 25 patients with epilepsy, irrespective of age group and not on any medication or supplements that may affect the results. BMD was measured using dual X-ray absorptiometry and was reported as *T*-score and *Z*-score. Urine hydroxyproline was estimated colorimetrically by modified Neuman and Logan method. Results were compared with age and sex matched apparently healthy controls. Bone mineral density was found to be significantly reduced ($P< 0.05$) in the study group. However, urine hydroxyproline was found to be near normal. Our results indicate that reduced bone mineralization is prevalent and a significant health concern in patients with epilepsy. Also antiepileptic drugs effects bone metabolism adversely. Hence, effective screening is a must and based on that newer medications are to be considered.

P176

Variation in Glycated Hemoglobin Levels in the Thalassemia Trait Population- A Pilot Study

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Glycated Haemoglobin (A1c) is useful as a tool to monitor blood glucose levels or treatment efficacy provided the person has a normal hematological profile. The prevalence of thalassemia trait in the local population is known to be high as also incidence of diabetes. A pilot study was undertaken to observe the variation in A1c in traits as compared to people with normal hemoglobin electrophoresis. The study was conducted in KMC, Hospital Laboratory between Sep13- Aug 14. Of the total 167 requests for hemoglobin variant analysis by HPLC, 65.6% were adults (18-60years) and only 44 cases with relevant complete data were included. The males and females were grouped separately as normal (M1, F1) or trait (M2, F2) and comparative analysis was done between the groups. A1c eluted as a separate entity and deviations if any in these groups was observed. 27(61.4%) adults were interpreted as Thalassemia traits. Only M2 had significantly lower mean hemoglobin values while F1 and F2 were comparable. Mean HbA2 values in both M2 and F2 were significantly higher than the normal. Only one each of F1 and M1 had HbA1c<4 due to coexisting iron deficiency anaemia. 8.3% of M1 and 20% F1 had

A1c >6.5%. It was notable that all the traits (M2, F2 ; 100%) had their HbA1c values ranging between 4.1-6.0% irrespective of their age and glycemic status. Presence of iron deficiency and or hemolysis can give subnormal A1c values whereas in the presence of thalassemia trait the A1c values show perfect glycemic control.

P177

Prevalence of Hemoglobin Disorders in North Delhi Population

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Testing for hemoglobinopathies and thalassemias is required to determine, whether an individual is a carrier or not. We describe the RBC indices of 412 whole blood EDTA samples of various patients from north Delhi to study correlation between various hemoglobinopathies/thalassemias and their corresponding RBC indices. A total of 412 whole blood samples from 393 pregnant females and 19 males from North Delhi area were collected intravenously in a EDTA BD vacationers at Biodiagnostic Lab, Rohni, Delhi. The whole blood EDTA samples were pre diluted and analysed using fully automatic CE-HPLC on Bio-Rad. D-10 Analyser. Out of 412 blood samples, 378 were normal (91.74%), while 34 (32 females and 2 males) (8.26%) were abnormal. Among abnormal cases, we have detected 22 cases (5.33%) of thalassemia minor, 5 cases of HbD Punjab (5.33%), 2 cases (0.48%) of Hb E heterozygous, 3 (0.71%) cases of Hb J Meerut, 1 (0.25%) case of Hb Q India and 1 case (0.25%) of HbD Iran. RBC's indices were also studied. In thalassemia minor, RBC was increased, while MCV & MC H were decreased markedly and Hb was normal or decreased compared to normal. MCHC and RDW were unchanged. The cut off value of Hb A2 for diagnosing thalassemia minor was 4.0 %.In Hb D Punjab, Hb F was slightly increased, while MCV and MCH were slightly decreased and RBC, Hb, HCT, MCHC and RDW and A_2 were unchanged as compared to normal. A0 was correspondingly decreased. In Hb J Meerut, RDW was increased slightly, while Hb, MCV, MCH, MCHC were decreased slightly compared to normal, while RBC, HCT, Hb F, Hb A_2 were unchanged with decrease in Hb A0 and increase in Hb J. In Hb E heterozygous, MCH was decreased slightly, Hb A0 was decreased significantly with increase in A2+E (about 30%), compared to normal. RBC, Hb, HCT, MCHC and RDW were unchanged. In Hb Q India, RBC, Hb, HCT, MCV, MCH, MCHC and RDW, Hb F and A2 were unaltered, while Hb A0 (70 %) was decreased compared to normal. In HbD Iran, RBC and A0 were decreased, while A2 + D Iran (>40%) was increased compared to normal. HbF was unchanged; while rest of the RBC indices was well within reference range.The occurence of hemoglobin disorders in north Delhi population were studied.

P178

Urinary Glycosaminoglycans - A Marker for MPS

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Mucopolysaccharidoses (MPS), a group of inherited lysosomal disorders are asssociated with defects in glycosaminoglycan (GAGs) metabolism. In addition to the characteristic clinical findings they may present with an increase in urinary GAGs excretion.The objective of the study was to optimize urinary GAGs estimation in a routine laboratory.We optimized and validated urinary GAG estimation using dimethylmethylene blue dye binding method. Age matched reference ranges were established in 145 healthy subjects ranging from neonates to adults. Subsequently screening test is being offered in our laboratory since October 2012. A detailed clinical history and a random urine sample is obtained from the suspected patients. The GAGs are reported as mg/mmol of creatinine and the results are interpreted with age matched reference ranges. Amongst healthy indiviudals GAG excreation decreases with an increase in age wherein children excrete atleast more than 3 times the adult range (1.5 – 5.1 mg/mmole creat). Thirty patients with developmental delay, coarse facies, bone defects etc were referred for screening. The suspected patients ranged from 9 days – 26 years. A variable increase (1.2-5.3 fold) in GAG excretion when compared with age matched reference range was seen 66% of patients. These patients were further referred for MPS enzyme assays for confirmation. Age matched reference ranges are required to interpret the GAG results. Excretion of GAG amongst MPS patients varies remarkably depending on the type and severity of the disease. GAG estimation is the first line screening test to rule out MPS and the screen positive patients need to be further worked up for specific enzyme analysis.

P179

Study of *IS6110* and *mpb-64* Mycobacterial DNA Sequences in CSF for the Early Detection of Tuberculosis

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Tubercular meningitis (TBM) involves the central nervous system (CNS) and is one of the most severe forms of extra-

pulmonary tuberculosis. Rapid detection of the causative organism (Mycobacterium tubercular Bacilli) is of paramount importance in TBM as the disease can be fatal and clinical outcome depends heavily on the stage at which treatment is initiated. Diagnosis of TBM is presumptive and is based on clinical symptoms, neurological signs, cerebrospinal fluid (CSF) findings, CT scans and the response to anti-tubercular drugs. Rapid techniques based on nucleic acid amplification such as PCR are more sensitive and specific as they attempt to detect specific DNA sequences of the organism. Aim was to study the mycobacterial DNA sequences for the early detection of MTB complex.This study comprised of the fifty patients of Tubercular meningitis admitted in Medical wards of SMI Hospital, Dehradun and fifteen patients as control (non-TBM). All collected specimens were subjected for molecular analysis using *IS6110* and *mpb-64* genes. Adenosine deaminase activity was also measured. Out of 50 clinical specimens, 29(58%) came positive for TB PCR, *IS6110* positive in 26 (52%) and *mpb-64* positive in 20 (40%). 17 specimens came positive by both the targets but 9 specimens alone came positive only by *IS6110* gene, and the same came negative for *mpb-64* gene, and 3 specimens came positive for *mpb-64* gene and the same were negative for *IS6110* gene. 2 specimens came positive for AFB smears and33 specimens have ADA activity >10 (IU/), the overall results gave a sensitivity of (58%) and specificity of (93.3%) for the diagnosis of TBM. The results from PCR, ADA activity and AFB staining could be helpful in prevention and treatment of tubercular patients.

P180

Biochemical Characterization of Methyltransferase Gene Involved in Tuberculostearic Acid Biosynthesis in *Mycobacterium tuberculosis*

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The precise knowledge on mycobacteria–host cell interactions are also a requirement for the successful control of TB. Emergence of multi-drug resistant tuberculosis (MDR-TB), extremely drug resistant tuberculosis (XDR-TB) and totally drug resistant tuberculosis (TDR-TB) enhances deadly effect of tuberculosis. Clearly in the addition to the improvement of human population welfare, there is an intensive need for development of new vaccines. Biochemical Characterization of methyltransferase gene involved in tuberculostearic acid biosynthesis in *Mycobacterium tuberculosis*". Biochemically characterized of this purified protein showed it to be involved in the methyltransferase activity. Methyltransferase activity checked under various conditions was then quantitated on the basis of this standard parameter. The biochemical characterization of this purified protein showed it to be involved in the methyltransferase activity. We identified of methyltransferase gene in *M. tuberculosis* H37Rv as the enzyme capable of *in vitro* biosynthesis of tuberculostearic acid. Studies are in progress to understand the physiological significance of these proteins in *M. tuberculosis* H37Rv. Identification of relevant in vivo targets of this methyltransferase gene will provide us the supportable information about the host pathways that are subverted and thus suggest a role for these factors in the pathogenesis of *M. tuberculosis.* This could open new approaches for the development of targets for new drugs and vaccines against TB.

P181

Molecular Quantification of BCR-ABL/ABL Ratio in CML & Its Usefulness in the Prognosis & Evolution of Disease

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Chronic myelogenous leukemia (CML) is a myeloproliferative neoplasm that originates in abnormal pluripotent bone marrow stem cell & is associated with the BCR ABL1 fusion gene located in the Philadelphia chromosome. Molecular diagnosis is done by detecting BCR-ABL fusion transcripts which is also helpful in the identification of various molecular subtypes on the basis of breakpoint cluster regions involved in the translocation. This was a data based study & a total of 90 patients were enrolled in the study. The molecular diagnosis was done on Real Time PCR in a stepwise manner using commercial kits. Firstly RNA was extracted from blood kits, then cDNA was synthesized which was subjected to quantitative PCR & the BCR-ABL/ABL Ratio was calculated. Out of the 90 patients who reported with mild to moderate leucocytosis Philadelphia chromosome was not detected in 14 patients, 18 patients were with negative BCR-ABL/ABL Ratio & 58 patients were found to be positive with mean value of 36 .17±7.43 of the ratio which decreased with the treatment. Molecular detection of the BCR-ABL/ABL Ratio is an important tool for assessing response to therapy & in developing more effective therapeutic modalities.

P182

Post-transcriptional Regulation of Genes by Non-coding RNA in *Neisseria gonorrhoeae*- an Obligate Human Pathogen

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Small non-coding RNAs (sRNAs) play an important role in bacterial gene expression and regulation. The knowledge of sRNA in *Neisseria gonorrhoeae* is scarce despite of its clinical significance. We utilized the available RNA-seq data under aerobic and anaerobic condition to identify non-coding RNA. We further identified sRNAs which are differentially expressed under anaerobic condition and their mRNA targets.The objective if the study was identification of non-coding RNA under aerobic / anaerobic conditions and their effect on expression of mRNA targets. The normalized reads (RPKM) under aerobic and anaerobic condition were compared and a three-fold or greater difference in the expression level of sRNAs reported as differentially expressed sRNA. sRNA targets were found using online available tools.We further predicted the sRNA-mRNA interactions using intaRNA tool.We identified ten sRNAs which were differentially expressed under anaerobic condition, physiologically important stage during infection. We further identified mRNA targets of these sRNAs based on deep sequencing of *N .gonorrhoeae* transcriptome under aerobic and anaerobic condition and found several sRNAs which target genes involved in energy metabolism processes, stress response and various other networks. Our results provide new insights into the post-transcriptional regulation of genes by sRNAs.

P183

Biointermittent Shedding of Tubercul Bacilli - Clinical Significance in Molecular Diagnosis and Prognosis of the Disease

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Urine is increasingly being investigated as a convenient clinical sample for the identification of mycobacterial products for the diagnosis of tuberculosis. The incidence of genito-urinary tuberculosis has markedly decreased in the Western part of the globe but the situation has not changed much in developing countries. Urine is increasingly being investigated as a suitable clinical specimen for the detection of mycobacterium tuberculosis for the diagnosis of tuberculosis. The aim of the present study was to evaluate a PCR assay for the rapid detection of Mycobacterium tuberculosis (MTB) in urine and comparison of the sensitivity of PCR with ZN staining. The single and multiple urine sampling were also studied in order to know the efficacy of the PCR on intermittent shedding of bacteria. Study includes the multiple sampling of the urine specimens for the diagnosis of urinary tuberculosis. Out of 48 specimens processed, 12 came positive for MTC by nested PCR, where as 1 showed AFB, when processed for ZN staining. Out of the 12 specimens, which came positive by PCR it was seen that in three positive cases, when only 1st void morning urine was processed for PCR it came negative, while the same when concentrated came positive. A case only came positive for ZN. *Mycobacterium tuberculosis* is found to be excreted intermittently in the urine of infected patients, and single specimens were more likely to be false negative than a 24-hours sampling. The best method appeared to be the concentration of a large volume of urine, for instance 11ml concentrated to 2 ml. Various advantages of tuberculosis PCR includes, non invasive; non infectious sample source, as urine is not infectious for most human pathogens. By the usage of NAATs, *Mycobacterium tuberculosis* can be detected within 3-4 hours of duration. As studied, it is very significant to concentrate the bacilli load, as mycobacterium tuberculosis may be missing in first morning urine or shed in urine. When multiple sampling is done, it is also important to have minimum bacilli load for the PCR though sensitive enough.

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Study of Zinc, Copper and Ferritin in Patients with Thalassemia and Sickle Cell Anaemia

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Repeated blood transfusion in patients with thalassemia and sickle cell anaemia leads to ferritin overload and micronutrient alteration.The aim of study was to investigate serum zinc, copper and find their correlation with serum ferritin in patients diagnosed as thalassemia and sickle cell anaemia. This observational study is carried out in the thalassemia ward of Paediatrics Department and lab investigation carried out in the Dept.of Biochemistry, A.M.C.H from June to August 2014. Total 30 cases of thalassemia and 20 cases of sickle cell anaemia were enrolled for study. 3 ml sample collected in SEV. Serum copper and zinc estimated in semiauto analyzer by colorimetric method and ferritin estimated in Immulite 1000 by chemiluminiscence method. In patients with thalassemia ferritin showed negative correlation with zinc ($P< 0.05$) and positive correlation with copper ($P< 0.05$) respectively which are statistically significant whereas in sickle cell anaemia cases ferritin showed positive correlation with both zinc ($P>0.05$ which is not statistically

significant) and copper (P<0.05 which is statistically significant). This study reveals that with raised level of ferritin hypozincemia is common in thalassemia patients but in sickle cell anaemia there are varying results. However in both the diseases copper level is increased.

P185

Comparative Study of the Status of Vitamin D_3 in Young Office Working Women and Housewives in Udaipur, Rajasthan

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Vitamin D_3 deficiency continues to be an unrecognized epidemic in many populations around the world. Vitamin D is important for the absorption of calcium, and bone formation & maintenance. The objective of study was evaluation of the prevalence of low vitamin D levels (insufficiency and deficiency) in female office workers and housewife's in Geetanjali Medical College and Hospital, Udaipur. This study was carried out in Geetanjali Hospital and Medical College, a retrospective study conducted on 50 working and 50 housewife's subjects between age group 18-32 years. Body mass index (BMI) was calculated. Serum calcium, serum phosphorous, and alkaline phosphatase were determined by a fully-auto-analyzer. The serum level of vitamin D_3 was measured using the electro chemiluminescence immunoassay methodology. The difference in the BMI between the office workers and housewife's was statistically not significant. Vitamin $D_3$was 18.72 ± 6.97ng/ml (SEM0.987) in office worker's and 9.94 ± 6.14 ng/ml (SEM 0.869) in housewife's with a highly significant (P<0.0001) statistical difference. In all subjects low vitamin D_3 levels were found. An urgent awareness with treatment of this deficiency must be undertaken to prevent any future consequences of vitamin D deficiency in these young healthy females.

P186

Study of Serum Zinc Level in Undernourished Children

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Protein energy malnutrition is one of the most common health problems among children of developing countries, including India. Growth retardation due to this condition occurs in children of post weaning age due to dietary deficiency of specific nutrients like zinc. Zinc has central role in cellular growth and differentiation. The aim was to compare serum zinc levels of healthy and undernourished children (1-5years). Institutional based case control study was done on 100 children(age 1-5 years), 50 children were undernourished (classified as per WHO) while 50 healthy childrenattending Pediatrics OPD for immunisation in Rohilkhand medical college and Hospital, Bareilly, Uttar Pradesh, from 1st January 2014 to 31st August 2014, were taken as control. Estimation of serum zinc by semiautoanalyzer using a commercial kit was done.The mean serum zinc levels of 50 healthy children were found to be within normal limit while mean serum zinc level of 50 undernourished children was significantly decreased. Statistical analysis of data was done by calculating P-value with analysis of variance of serum zinc level for degree of malnutrition. It showed that serum zinc level of undernourished children is significantly low (P value = 0.001).There is a significant difference of serum zinc levels between healthy and undernourished children.This signifies a proper replacement of zinc as part of management of undernutrition.

P187

Comparative Study of Vitamin D Level in Rheumatoid Arthritis Patients and Healthy Control Subjects

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Low level of vitamin D (25-hydroxyvitamin D) is very much common in Indian population despite its sunny climate. Skin complexion, poor sun exposure, vegetarian food habits and lack of vitamin D food fortification programme in the country explain the high prevalence of Vitamin D Deficiency. Emerging evidence suggests that vitamin D plays an important role in immune regulation. Preliminary studies suggest that low levels of vitamin D may be common in rheumatoid arthritis (RA). We aimed to find out the level of Vitamin D in Rheumatoid arthritis patients and in Healthy control subjects and compare the level of Vitamin D between the two groups and to analyse the association of vitamin D level with disease activity and disability. This is a case control cross-sectional study, which included 50 patients suffering from Rheumatoid Arthritis (study group) and 50 healthy volunteers (control group). Members of both sexes and age between 25-75 years were included in study. Both groups were comparable on age, sex, socioeconomic status and other sociodemographic variables. Vitamin D level was assessed by HPLC method and UV detector at 265 nm. Rheumatoid arthritis disease activity and disability were assessed by physician with the help of disease activity score (DAS 28). A statistically significant decreased concentration of serum vitamin D_3 was found in RA patients as compared to that of normal control group.

P188

Role of Trace Elements in the Formation of Gall Stones

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Recent studies have defined the role of trace elements Fe, Ca, Zn and Cu in the formation of gallstones. Iron acts as a cofactor for niric oxide synthase which synthesizes NO and is important for maintenance of the basal gallbladder tone and normal relaxation. So, deficiencies of serum Fe and calcium can lead to increased risk of gallstone disease.The present study was planned to analyze the exact role of serum iron and calcium in the pathogenesis of gallstone disease and to assess the relationship of biliary cholesterol supersaturation with levels of serum Fe and calcium. Total 100 patients suffering from cholelithiasis were included in the study and were divided into four groups: Group A & A1 [included patients with normal serum Fe & calcium respectively (controls)] & group B & B1 [included patients with iron & calcium deficiency respectively (cases)]. Five ml of blood was taken from the subjects. Bile cholesterol levels were raised in the cases as compared to the controls. Low serum iron, causes defective hepatic cholesterol metabolism and more stasis of bile because of decreased motility of gallbladder and leads to increased precipitation of cholesterol and hence gallstone formation. Also calcium deficiency causes deranged function of NOS which produces relaxation of gallbladder. Hence gallbladder stasis occurs which subsequently increases crystal formation in bile. Thus, Fe and calcium deficiency is associated with increased super-saturation of bile followed by increased incidence of gallstone formation.

P189

Association of Zinc with Bone Metabolism in Inflammatory Bowel Disease Patients

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Bone demineralization is a significant problem in Inflammatory Bowel Disease. Contributing factors including inadequate nutrition, corticosteroid, and decreased physical activity. Trace elements play an important role in the growth development and maintenance of bones. The aim of our study was to assess the relationship between the serum Zinc level and the bone Mineral indexes in Inflammatory Bowel Disease patients. Forty two newly diagnosed patients of Inflammatory Bowel Disease and forty healthy Controls of both gender ranging in age from 19-50 years were included in the study. Fasting blood samples were processed for following biochemical parameters– Serum Calcium, Phosphorus, Vitamin D, Parathyroid Hormone and Zinc. The subjects were evaluated for Bone Mineral Density (g/cm^2) using Dual Energy X-ray Absorptiometry scan and T score was calculated to assess Osteoporosis. Student's unpaired t-test, one way ANOVA and Pearson correlation tests were used for statistical analysis. Inflammatory Bowel Disease patients had significantly lower Bone Mineral Density than the Controls. Bone Mineral Density values were not different between the subtypes Crohn's Disease and Ulcerative Colitis. Though Ulcerative Colitis and Crohn's Disease patients had significantly lower Bone Mineral Density than the Controls. Low Zinc level was observed in 50% of Osteopenic and 80% of Osteoporotic subjects. Zinc level was positively correlated with Bone Mineral Density(r=0.24) and Vitamin D (r=0.25). Patients with Inflammatory Bowel Disease are more prone to develop metabolic bone disease. Along with other nutrients supplement Zinc should be added to prevent bone loss.

P190

Status of Antioxidant Vitamin (Vit C & Vit E) in Diabetic Patients with or without Dyslipidemia

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Diabetes is the commonest metabolic disorder affecting the people all over the world, characterized by hyperglycemia, lipoprotein abnormalities and altered intermediary metabolism of major food substrates. Hyperglycemia in DM is associated with increased lipid peroxidation and excessive production of free radicals which lead to oxidative stress by autoxidation of glucose. It is an important pathogenic mechanism in the development of diabetes and its complications. The aim was to examine serum level of vitamin. C and vitamin E in diabetes with or without dyslipidemia.The research work included 100 patients with type 2 diabetes mellitus (age group 30-60 years). Fasting blood samples collected using asceptic technique were evaluated for serum levels of the fasting blood glucose, triglycerides, total cholesterol, HDL, LDL,Vitamin C and Vitamin E by spectrophotometric method. Data were analyzed using Microsoft Excel 2007 and results were expressed as Mean±SD. It was observed that serum level of vitamin. C and vitamin E are significantly low ($P<0.05$) in diabetic patients with dyslipidemia as compared to patients without dyslipidemia. This indicates the protective role of antioxidants in development of dyslipidemia in diabetics.

P191

Study of the Role of Copper, Zinc & Magnesium in Diabetic Nephropathy

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Diabetic nephropathy is a complication of diabetes mellitus. A copper, zinc and magnesium important nutrient that is involved in various physiological metabolisms. This present study investigates the relation of copper, zinc and magnesium in diabetic nephropathy cases to establish possible results. Thirty healthy no diabetic subjects were studied for comparative analysis. Thirty diabetic Blood samples were collected from both cases and controls for determination of FBS, PPBS, HbA1c, copper, zinc and magnesium levels. The mean concentrations of FBS, PPBS, HbA1c, cases were significantly higher than that of controls. The mean magnesium levels of cases (1.80 ± 0.29 meq/L) were significantly lower than controls 2.40 ± 0.20 meq/L ($P< 0.05$). But the mean copper levels of cases, 150.42 ± 5.40 μg/dl, shows no significant difference with controls, 156.6 ± 5.70 μg/dl, ($P> 0.05$). The mean zinc levels of cases, 55.45 ± 33.53 μg/dl, shows no significant difference with controls, 60.73 ± 12.3 μg/dl ($P> 0.05$). The findings in the present study suggest that hypomagnesaemia may be linked with development of diabetic nephropathy.

P192

Vitamin D Insufficiency: Risk Factor for End Stage Renal Disease in Chronic Kidney Disease (CKD) Patients

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Vitamin D Insufficiency in general population and in CKD Patients is based mainly on effects of Vitamin D on calcium homeostasis and bone health. It is associated with proteinuria and thus, contributes as a risk factor for End Stage Renal Disease in CKD patients.The present study was designed to assess the level of Vitamin D with advanced stages of CKD.The study was carried out in 40 CKD Patientsin which calculated was GFR<60 ml/min/1.73m^2. CKD Stages were divided into six categories based on GFR (Stage I = 90 ml/min/1.73m^2, Stage II 60 – 89 ml/min/1.73m^2, Stage IIIa 45-59 ml/min/1.73m^2, Stage IIIb 30 – 44 ml/min/1.73m^2, Stage IV 15 - 29 ml/min/1.73m^2, Stage V < 15 ml/min/1.73m^2). For the purpose of study, Stage I and Stage II were excluded as GFR calculated was > 60 ml/min/1.73m^2. Routine investigations were performed. Vitamin D levels of these patients were calculated using ELISA. Also, Statistical Differences in variables were compared using one way analysis of variance (ANOVA) and unpaired Student's t test. Mean Vitamin D levels were significantly lower according to severity of renal impairment (CKD Stage IIIa : 28.32 ± 11.03 ng/ml; CKD IIIb : 26.26 ± 10.27 ng/ml; CKD Stage IV : 23.15 ± 11.85 ng/ml; CKD Stage V : 18.62 ± 10.24 ng/ml) ($P< 0.001$). The prevalence of Vitamin D insufficiency and deficiency was 60.4%, 65.2%, 70% and 86.7% in Stage IIIa, IIIb, IV and V respectively.This study demonstrates that Vitamin D insufficiency and deficiency are associated with level of kidney function in CKD Patients especially advanced stage renal disease.

P193

Study of Trace Elements in Liver Cirrhosis Patients and their Role in Prognosis of Disease

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The aim was to evaluate trace elements in patients with Liver Cirrhosis and assess their association with severity of the disease. 150 Cirrhotic subjects of either sex ranging in age from 20-70 years (mean± SD 44.04±8.57 years) were included in the study and the results were compared with 50, age (mean ± SD 43.14 ± 9.37 years) and sex matched healthy control subjects. All Cirrhotic subjects were assessed for severity of disease as Mild (Child A), Moderate (Child B) and Severe (Child C) as per Child Pugh classification. Routine investigations were done and trace elements were analyzed on Atomic Absorption Spectrophotometer. Serum level of Copper content was found significantly increased in Cirrhotic patients as compared to control group. Whereas Serum Zinc, Selenium and Magnesium levels were significantly decreased in Cirrhotic subjects as compared to healthy Controls. Trace elements were compared with severity of liver cirrhosis. Serum Copper concentration was slightly increased in patients with more severe clinical state of liver cirrhosis, however mean level difference of Copper among the Child Pugh groups were statistically not significant. Moreover there was no significant correlation between Copper and Child Pugh Score. However Copper showed a significant negative correlation with Zinc. The Serum Zinc, Magnesium and Selenium levels were significantly decreased with advancement of liver disease (Child B and C) as compared to early stage of liver cirrhosis and showed a significant negative correlation with Child Pugh Score. Micronutrients supplementation in liver cirrhotic patient can prevent progression of disease and development of complications.

P194

Serum Calcium in Schizophrenic Patients: A Comparative Analytical Study

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Schizophrenia is a disabling brain disorder and the major contributors to the global burden of brain diseases. Recently, several studies have highlighted the alteration in serum Calcium (Ca) levels in schizophrenic patients; however, show a variety of results. Because of this recent heightened interest, variability in results and scarcity of documentation on this issue in Indian literature, we are inspired to undertake this study with objective to compare Serum Ca levels in patients having schizophrenia with the control group and find out the relationship between serum Ca and stage of disorder (acute and chronic) if any. The study was conducted in Department of Biochemistry and Psychiatric Centre at SMS Medical College, Jaipur. Two groups of 150 each participated in the study. Group 1: The diagnosed patients of schizophrenia. Diagnosis was confirmed by one senior consultant according to ICD-10 criteria. On the basis of duration, symptoms of illness and PANSS score, each of schizophrenic was further categorized as, in the Acute (n=79) and Chronic (n=71). Group 2: Age & sex matched 150 control subjects from general population. The estimation of serum Ca was carried out on a fully automated chemistry analyzer (OLYMPUS AU 400) by Arsenazo Π method. 'P' Value < 0.05 was taken as significant. Serum Ca level was 8.94 ± 0.44 mg / dl in case group while it was 9.92 ± 0.42 mg/dl in control group. This difference was significant (P = 0.000). Ca level was found statistically significant (P = 0.028) between acute (9.01 ± 0.48 mg/dl) and chronic cases (8.85 ± 0.37 mg/dl). We conclude that there was significantly lower level of Ca which may be because of some changed metabolism of Ca in long duration schizophrenic patients by altering some hormonal axis or substantial change in diet. Ca supplementation may be beneficial.

P195

Estimation of MDA and Vitamin C in Protein Energy Malnutrition (PEM) in Children Age Group 6 Months to 5 Years

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Malnutrition is the cellular imbalance between supply of nutrients and energy & the body demand for them to ensure growth, maintenance and specific functions.The present study was undertaken to evaluate the role of oxidative stress in PEM children. 60 PEM children in the age group of 6 months to 5 years were selected and compared to 60 healthy age & sex matched controls. The degree of lipid peroxidation was measured in terms of malondialdehyde (MDA) by the thiobarbituric acid test & Antioxidant vitamin viz. Vitamin C was measured by Ayekyaw Method 1978; to assess the status of oxidative stress in malnourished children in comparision to healthy controls.There was significant increase in the product of lipid peroxidation as compared to controls. On the other hand, there was significant decrease in the serum vitamin C levels. Thus it can be concluded that PEM induces a state of oxidative stress which produces reactive oxygen species (ROS). These ROS cause lipid peroxidation that leads to increased MDA concentration. Decreased antioxidants further aggravate the oxidative stress.

P196

Vitamin D Status in Female Patients in Punjab

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Vitamin D is responsible for maintaining bone health and calcium homeostasis. Recently a relationship between low vitamin D and disease like cancer, CVD, diabetes and multiple sclerosis has been reported. Several factors like exposure to sun light, dietary habits and cultural factors affect its levels. High prevalence of Vitamin D deficiency has been reported in India and we expect females to be affected more as they remain indoor or cover their body due to cosmetic/cultural reasons so we studied female patients visiting tertiary care hospital. The objective of the study was to screen all female patients presented with vitamin D deficiency symptoms to observe vitamin status with age. In tertiary care hospital, 5110 females (symptoms like backache, unexplained muscle pain and fatigue) reported for vitamin D investigation in the year 2013. They were divided into four age (years) groups, Group I (0-18), Group II (19-40), Group III (41-60) and Group

IV >60 . 25(OH) D was estimated on Cobas 6000 (Roche). Vitamin D levels (ng/ml) classified as: severe deficient <4, deficient <20, insufficient 20-30, sufficient 30-70 and excess >70ng/ml. Maximum females reported for Vitamin D investigation were in groups II & III. 48.2% females were having deficient levels. Only 29% females had sufficient levels. We further observed that majority of females were vitamin D deficient in all groups. But deficiency decreased significantly with age (61.7% in group I to 36.6% in group IV) whereas in sufficient levels increasing trend was observed (group I 19.3% to 37.5% group IV, P<0.01).We observed vitamin D deficiency decreases with age, may be they are already on treatment as vitamin D supplement is recommended for all type of chronic illness. More of old females were expected to be deficient, but it was reverse, may be due to awareness and supplementation. Rather more of young females were found deficient as majority avoid sun exposure due to cosmetic/cultural reasons.

P197

Polyketide Synthase Based Identification of *Aspergilli* and Sequence Variation Analysis of *pksA* in *A flavus*

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A. flavus, A. fumigatus, and *A niger* are implicated in allergic and invasive forms of Aspergillosis. Specific detection methods are needed for appropriate fungal therapy for better outcome Polyketide biosynthetic pathway in aspergilli is of great scientific interest. Sequence variations in important genes of aflatoxin biosynthetic pathway can contribute to understanding of the toxigenicity and the diversity of polyketides of pharmaceutical importance they secrete. Phylogeny of PKS gene sequences in combination with sequence variation studies in pksA gene are examined and used for differential detection of *A. flavus, A. fumigatus* and *A. niger*. The objectives of the study phylogenetic relationship of pks in important *Aspergilli* for development of diagnostics and examining the molecular variations in the pksA gene in *A. flavus* isolates of Indian origin. PKS protein sequences from *A. fumigatus, A. flavus* and *A. niger* were retrieved, aligned and phylogenetic analysis was performed. Genus specific degenerate primers were designed, synthesized and used in Multiplex PCR for the detection of *Aspergilli*. pksA gene from a total of 39 isolates of *Aspergillius flavus* were amplified using nested primers and sequenced. These isolates were also extracted for aflatoxins and were tested by TLC. Unique and degenerate primers can detect all the three *A. fumigatus, A. flavus* and *A. niger*si multaneously and uniquely. Sequence variations in pksA gene of *A. flavus* isolates of Indian origin showed different polymorphisms and deletions in gene, which can be linked to atoxigenicity. This work also reports the molecular characterization and toxigenic profile of geographically distinct *Aspergillus flavus* isolates of Indian origin. Phylogenetic analysis and domain diversity analysis of *Aspergillus* PKS enzymes resulted in development of specific and differential detection primers and probes for *Aspergilli.* Few notable sequence variations in pksA gene of *Aspergillus flavus* were observed to be associated with toxigenicity. These isolates can be further examined and explored for applications.

P198

Dysregulation of Labile Plasma Iron Activity and Ferric Reducing Antioxidant Status in Development of Anemia

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Reactive oxygen species are involved in development of various nutritional diseases and disorders while anemia is widely prevalent amongst women and children in developing countries. Since no data is available on labile plasma iron activity and their association with reactive oxygen species in anemic adolescent girls. However, oxidative stress markers, labile plasma iron activity and iron status of anemic adolescent girls were assessed. 100 adolescent girls (Age range 13-16 years) were randomly screened from the Government girls Junior School, Jaipur. Subjects were categorized into two groups based on hemoglobin status i.e. Non anemic (n=50; Hemoglobin >120 g/L) and anemic (n=50; hemoglobin <120g/L). Fasting blood samples were collected after getting an ethical clearance. Lipid peroxidation, superoxide dismutase, vitamin C, vitamin E, free radical antioxidant potential, labile plasma iron activity, ceruloplasmin, serum iron and urinary copper levels were analyzed in both the groups using the standard methodology. Hemoglobin estimation was done by Drabkin's method. Statistical analysis was done by SPSS software and P values <0.05 were deemed statistically significant. In case of anemic adolescent girls, Vitamin C (0.86±0.14 mg/dl), free radical antioxidant potential (860.5±78.4μM/L), superoxide dismutase (1.38±0.55U/ml) and serum iron (8.54 ±2.12μM/L) levels were significantly decreased (P<0.05) while lipid peroxidation (6.47±1.40nM/ml) and urinary copper (36.9±6.85μM/L) levels were significantly increased (P<0.05). Vitamin E (24.22±1.56μM/L) and ceruloplasmin (69.8±18.22mg/dl) levels were non-significant (P>0.05). The levels of Vitamin C (1.29±0.52 mg/dl), free radical antioxidant potential (1036±82.5μM/L), superoxide dismutase (2.48±0.44U/ml), serum iron (11.22 ± 1.32μM/L), lipid peroxidation (3.62±3.5nM/ml) and urinary copper (16.9±2.7μM/L) were assessed in non-anemic adolescent girls. Labile plasma iron activity was increased in anemic adolescent girls. Therefore increased activity of labile plasma iron suggests that the synergistic involvement of oxidative stress via Fenton reaction could be responsible in development of anemia.

Labile plasma iron activity and total antioxidant status could be early markers for assessment of anemia and thus, the future strategies can be planned to overcome the expression of labile plasma iron activity with the supplementation of macro and micronutrient in adolescent girls.

P199

A Rare Case of Cystine Type Stone in a 33 Year Old Male Patient

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Nephrolithiasis is a common condition affecting nearly 5 in 1000 persons. Factors responsible for stone formation include low fluid intake, excretion of excess quantity of stone components, absence of substances inhibiting stone formation and urinary pH. Here is presented a rare case of cystine type stone in a 33 year old male patient Urinary calculi analysis was done by semiquantitative titrimetric and colorimetric method for calcium, Oxalate, Phosphate, magnesium, ammonium, uric acid and cystine. The Kidney stone was further confirmed by Fourier Transform Infrared Spectroscopy (FTIR) method. Semiquantitative method gave 5% Calcium, 20% Oxalate, 5% Phosphate and 70% Cystine suggesting that stone is mainly Cystine type with calcium Oxalate and traces of calcium hydrogen phosphate (Brushite). FTIR method gave 100% cystine. Urine pH = 8.0, leukocytes = 15-20 / HPF {0 – 5}, Urine microscopy did not show any crystals. Mainly cystine type stone was confirmed which rare type is (1-2%). Relatively low limit of solubility 18 mg/dL is exceeded in many patients with cystinuria resulting in the formation cystine stones. Cystine stone becomes soluble when pH exceeds 7.4; hence its crystals were not visible in urine microscopy.

P200

Relationship Between PTH Levels and Serum Creatinine in CKD Patients

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Chronic kidney disease (CKD) is a chronic and progressive disease characterized by renal dysfunction due to the decrease of the glomerular filtration rate (GFR). Hyperparathyroidism—an elevation of parathyroid hormone (PTH) is a complication of CKD. Observational studies consistently report an increased relative risk of death in CKD patients who have PTH values at the extremes (less than 2 or greater than 9 times the upper normal limit of the assay). The present study is focussed to study the relationship between PTH levels and serum Creatinine/GFR in CKD patients.The present study included 150 patients who have been diagnosed as CKD patients. The patients included both males (75) and females (75) with an age group between 22yrs–70yrs. The measurements of creatinine & PTH are done on the serum in Unicel DXC860i. The GFR is calcutated by MDRD formula and using creatinine measured by Modified Jaffe's traceable to IDMS. PTH is analysed by CLIA.The main finding in the study was a significant raise in the PTH values with an increase in creatinine values. Statistically the increase in PTH is higher in males ($r = 0.557$, $P<0.001$) when compared to females ($r=0.121$,$P<0.001$). Progressive increases of PTH should be avoided and marked changes in PTH levels should trigger a response to avoid a future result outside the range. Monitoring trends is important for the detection and treatment of CKD. In patients with CKD it is reasonable to base the frequency of PTH monitoring on the presence and magnitude of abnormalities and rate of progression of CKD.

P201

Effect of Antioxidant Enzymes and MDA Level in Hypertensive Chronic Kidney Disease Patients

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Hypertension is one of the most important factors associated with the progression of both diabetic and non diabetic CKD (chronic kidney disease). In the general population showed that hypertension is a strong independent risk factor for ESRD (end stage renal disease). Chronic kidney disease (CKD) is defined as persistent kidney damage accompanied by a reduction in the glomerular filtration rate (GFR) and the presence of albuminuria. Total Antioxidant Capacity (TAC), Superoxide dismutase (SOD), Catalase, Malondialdehyde (MDA), serum urea, serum creatinine and serum uric acid were assayed in 241 subjects In which 78 CKD patients with hypertension, 72 CKD patients without hypertension and 91 healthy controls.In our study, we found statistical significantly significant decreased level ($P<0.001$) of Total Antioxidant Capacity (TAC), Superoxide dismutase (SOD), Catalase and significant increase level ($P<0.001$) of malondialdehyde (MDA) in CKD with and without hypertension and also found deranged renal functions.The reduced activities of antioxidant enzymes status and increased production of malondialdehyde in the hypertensive patients confirms the presence of oxidative stress The data suggest that alteration in antioxidant

status and MDA in hypertensive CKD patients that binds support to role of oxidative stress in hypertensive patient.

P202

Study on Biochemical Changes Before and After Haemodialysis in Subjects of Renal Failure

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The kidney eliminates many molecules some of which have been implicated as uraemic toxins. These include urea, creatinine, potassium, hydrogen and phosphate ions.These Molecules with wide ranges of molecular sizes, lipid solubility and protein bindings behave differently across the dialysis membrane, so, the comparative study of certain known and still unknown particles may have immense role to assess the successfulness of dialysis and prognostic fate of patients of chronic renal failure. A large number of people are undergoing routine dialysis per year due to various causes of renal failure. In our study we have selected the patients of renal failure caused by Diabetes Mellitus with an object to find out a correlation between pre-dialytic and post-dialytic phases of dialysis. The study has been conducted in the department of Biochemistry in association with Department of Medicine, MGM Medical College, Kishanganj, Bihar, India. Plasma glucose, serum creatinine, urea, electrolytes, LFT, Thyroid profile have been performed before and after dialysis. Results will be discussed.

P203

Chronomics of Circulating Plasma Lipid Peroxides and Antioxidant Enzymes and Other Small Molecules in Renal Stone Formers

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The circadian periodicity of lipid peroxides and antioxidant defense mechanisms may change in renal stone formers and may prove to be of physiological significance in understanding the role of oxidants and antioxidants in etiopathogenesis, prevention and treatment of urolithiasis. The circadian periodicity of circulating plasma lipid peroxides in terms of malondialdehyde (MDA), antioxidant enzymes as Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione Reductase (GR) and other biochemical variables as Ascorbic acid and Uric acid levels were studied under near tropical conditions in 50 healthy volunteers (age: 20 to 40 years) and 50 renal stone formers, admitted in Surgical wards of Shri Mahant Indiresh Hospital, of similar age group with a diurnal activity from 06:30 to about 22:00 and nocturnal rest. Blood samples were collected every 6 hours for 24 hours under standardized conditions beginning at 06:00. All studied variables were quantified and enzyme activities were measured with spectrophotometric procedures. A marked circadian variation was detected in all studied variables in healthy Indians and renal stone formers by population-mean cosinor analysis (almost invariably $P<0.001$). Changes as a function of time for studied variables were observed in the MESOR, circadian amplitude and/or acrophase of many of the variables examined. A tendency for the amplitude to decrease and the acrophase to advance in renal stone formers was noticed exhibiting the role of oxidative stress in such patients which warrants further confirmation. Mapping the broader time structure of different physiological variables in renal stone formers with multi-frequency components of oxidants and antioxidants will be of significance in understanding the mechanism of crystal growth in renal tubules, and thus its prevention.

P204

Analysis of Thyroid Function Test Results in Critically ill Patient

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This becomes a challenge to the laboratory consultants to validate TFT results of critically ill patients as their results are abnormally altered. These alterations in a euthyroid, systematically ill patients are referred to as "Euthyroid sick syndrome". There are selected patients in ICU who actually have biochemically perturbed thyroid function, either hypothyroidism or hyperthyroidism. The aim was to analyse TFT results of critically-ill patients, their clinical status & past history of any thyroid disease & find out the pattern of TFT in true thyroidal disorders.TFT of patients admitted in ICU & MICU of KIMS were analysed in the Central Laboratory, done in the backdrop of clinical assessment of thyroid status on admission & in the past. The data was analysed & various trends were analysed using correlation coefficient analysis & student's 't' test. Most patients had normal TSH level but altered TT_3 & TT_4. Majority had low TT_3 & TT_4 levels (mean 4.0) with normal TSH. FT_3 was increased and FT_4 normal with normal TSH in one group. Few had lowered FT_3 & normal FT_4 with normal TSH. From the results analysed it was concluded that if past history of thyroid disease is ruled out then most TFT results should be repeated in patients once

they are out of the acute condition to assess their true thyroidal status. Thyroid function generally returns to normal as the acutce illness resolves. A consensus should be created among the physicians whether to perform TT_3 & TT_4 or perform FT_3 & FT_4 along with TSH levels.

P205

Increase in Glucose Concentration Interferes with Estimation of Electrolytes by Direct and Indirect Ion Selective Electrode Methods

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The estimation of electrolytes like sodium (Na^+), potassium (K^+) and chloride (Cl^-) using direct and indirect ion-selective electrodes (ISE) is a routine clinical laboratory practice. Interferents like proteins, triglycerides, drugs etc. are known to affect the results. The present study was designed to analyze the effects of increasing glucose concentrations on estimations of Na^+, K^+ and Cl^- by direct and indirect ISE. Pooled sera was mixed with glucose stock solution (2000 mg/dL) prepared in normal saline to obtain glucose concentrations ranging from ~100mg/dL to ~5000 mg/dL. Na^+, K^+ and Cl^- levels were estimated by direct (Combiline product, Eschweiler, Germany) and indirect ISE analyzers (Roche Diagnostics, Germany) and results were statistically analysed using ANOVA and Pearson's correlation. Similar experiment was also performed in 24hr urine sample from healthy subjects. Significant difference was observed between Na^+ and Cl^- measurements by direct and indirect ISE, with indirect ISE values being consistently higher than direct ISE. Besides this, significant difference was observed amongst Na^+ and Cl^- values from baseline values obtained by indirect ISE at glucose concentrations = 2486 mg/dL. However, no such difference was observed with direct ISE. Na^+ and Cl^- estimation by indirect ISE showed significant negative correlation with glucose concentration, more so, above ~1000 mg/dL. K^+, however, showed no significant difference with varying glucose. Similar results were observed in 24 hr urine samples with a significant difference observed amongst Na^+ and Cl^- values at =2000 mg/dL glucose. High glucose concentrations show significant negative interferencein estimation of Na^+ and Cl^- by indirect ISE in serum as well as urine.

P206

Direct Estimation of Ionized Calcium Results in Better Accuracy than Calculated Estimation

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Ionized calcium is the physiologically relevant component of blood calcium. However, direct measurement of ionized calcium is limited by difficulties in accurate analysis, lack of standardization, and need for special handling. Therefore, strategies have been developed to determine measurements that are more available and relatively inexpensive. This study compares the advantages and limitations of direct and calculated determinations of total and ionized calcium at different albumin concentrations. Randomly stratified serum samples (n=192) (every 10^{th} samples) submitted in indoor clinical biochemistry laboratory in PGIMER, Chandigarh over a period of two months were included in the study. The serum samples were then analyzed in Roche autoanalyzer for albumin, calcium, and ionized calcium simultaneously and were grouped into high (albumin >5.2g/dL), normal (albumin 3.5-5.2g/dL) and low (albumin<3.5g/dL) albumin groups. The formulas by Orrell et al, Berry et al and Payne et al were chosen for calculating albumin corrected calcium. The calculated ionized calcium (ICa_{calc}) was obtained by taking 50% of these values. Direct ionized calcium was measured by ion selective electrodes. Statistical difference between calculated and directly measured calcium was analysed using ANOVA followed by Tukey's post test in all the three groups. Correlation between z scores of calculated and measured calcium was measured using Pearson's correlation. A significant difference ($P<0.05$) was observed between calculated and directly measured total as well as ionized calcium. However, at normal albumin levels Total calcium by Payne et al showed agreement with measured total calcium. It was observed that a significant and high correlation between measured total calcium with albumin adjusted total calcium by all the three formulas in all the three groups. Whereas, very low and non-significant correlation was observed between z scores of measured and corrected ionized calcium. Corrected formulas do correlate with total calcium but lacks utility for predicting ionized calcium for which there is an immense need to evolve these formulas or these formulae should rather be abandoned for calculating ionized calcium.

P207

Significance of Ionized Calcium in Hypertension

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Objective was to asses the variations of mean serum total and ionized calcium levels in the patients of essential hypertension, and to compare them with the age and sex matched healthy normotensive controls. We also tried to observe the association of serum calcium (total and ionized) with essential hypertension. A total of 47 hypertensives and 28 normal control subjects were selected for the study. Blood pressure were measured and serum total and ionized calcium were estimated in them. Serum total calcium was estimated by O-Cresolphthalein Complexone Method inSemi-Automatic Analyzer. Serum ionized calcium was estimated by Electrolyte Analyzer (Ion Selective Electrode). Statistical analysis was done by SPSS 20 software. The mean serum total calcium level was decreased in hypertension patients than controls but the decrease was not significant (P= 0.96). Result also showed the mean ionized calcium level was decreased with advancing age in hypertensives than controls significantly (P<0.0001). Our study have distinctly shown that the mean ionized calcium level is negatively correlated with age in hypertension patients(r= -0.87, P<0.0001) but no correlation found in controls. Serum ionized calcium has a significant role in pathophysiology of hypertension. Ionized calcium may be estimated in essential hypertension patients to asses the prognosis. Calcium supplementation may improve hypertension.

P208

hsCRP in Pre-hypertension and Hypertension, a Prospective Study in Southern Asian Region

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Hypertension has turned into a leading cause of non-communicable disease, contributor to premature death in developed countries and the seventh in developing countries. In the regard of early diagnosis and better prognosis, the concept of pre-hypertension, (a systolic blood pressure of 120–139 mmHg and/or a diastolic blood pressure of 80– 89 mmHg) was introduced as the new guideline for the management of blood pressure by the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JnC-7).Among other factors inflammation may be a causative factor for development of Hypertension But the association is not very clear. Accordingly, we have designed our study to find any association of hsCRP with pre-hypertension and hypertension so that early prevention and control can help to avoid or delay the complications of hypertension. A total of 37 hypertensives, 30 pre-hypertensives and 31 age and sex matched healthy control subjects were selected for the study.Two BP readings were taken five minutes apart, on both arms, with a mercury sphygmomanometer. The estimation of serum hsCRP was doneon XL-600 Automatic Analyzer with the kit (ERBA Mannheim) based on the measurement of antigen-antibody reaction by the end-point Method.There is significant difference in systemic and diastolic blood pressure and hsCRP in between group study**.** In pre-hypertensive group hsCRP is correlated with diastolic blood pressure. Our results suggest a correlation exists between hsCRP and hypertension more significantly with pre-hypertension. So estimation of serum hsCRP in pre-hyertensive stage can prevent the occurance of hypertension and cardio vascular disorders thereby.

P209

Evaluation of Vitamin B12 Deficiency Amongst Preschool Children of East Delhi - A Hospital Based Study

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Nutritional anaemia in children contributes to a significant public health problem worldwide. Vitamin B12 is essential for the synthesis of S-adenosyl methionine which is involved in the metabolism of different proteins, phospholipids and neurotransmitters. There is paucity of data on the prevalence of Vitamin B12 deficiency in pre-school going children in India. Anemia is an important health concern worldwide, particularly in tropical and sub-tropical countries such as in India. The objective of the present study was to determine the prevalence of vitamin B12 deficiency in pre-school children of 6 to 60 months (5 years).Vitamin B12 assays on 528 children between 6 months to 5 years over a period of 2 years were reviewed. Individuals were considered deficient if vitamin B12 levels < 200 pg/mL and border line deficient if levels were < 350 pg/mL. Vitamin B12 deficiency was observed in 44.1% of the paediatric population. Most of the children belonged to the age group of 6-24 months. 52.5% children were found deficient when cut-off was taken < 200 pg/mL. Mean value of B12 observed in the study population was 188.96±93.1 pg/mL. No difference in B12 levels were observed between male and female children. Anemia is common in this population. Vitamin B12 status is an important predictor for plasma Hb concentration. Improving the status of this micronutrient may reduce the burden of childhood anemia in India.

P210

Blood Biochemical Markers for Endemic Fluorosis: A Case Control Study

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Fluoride in ground water is a major health problems not only Rajasthan but also worldwide. The prolonged exposure of fluoride can accumulate in the body and it may cause significant damage to health like dental and skeletal Fluorosis. In the present study we attempt to investigate biochemical markers for diagnosis of early Fluorosis. In the present study, 150 fluorotic patient (age 20 to 40 years) were recruited for the assessment of biochemical markers and 150 healthy age and sex matched controls were selected. Blood samples were taken for fluoride estimations, hematological test and oxidative stress markers. The concentration of fluoride and lipid per oxidation was increased while antioxidants levels SOD,CAT and glutathione were reduced in subjects as compared with the controls. In addition the number of WBC and RBC were changed along with the packed cell volume. The concentration of Hb and heart rate were also changed in subjects. On the basis of result it may conclude that fluoride causes increased lipid peroxide and decreased antioxidant levels and changed hematological profiles. The spectrum of analysis could be useful for the assessment of early Fluorosis as primary symptoms.

P211

Accuracy of Syndromic Disease Management and PCR Assay in Diagnosis of Vaginal Discharge in Women Visiting OPD of Obstetrics & Gynecology in Referral Hospital

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Overtreatment of sexually transmitted infections (STI) using clinical observations is now well established. This has huge economical and medical consequences and has led to development of resistant strains in the population due to lack of confirmed and accurate diagnosis. The aim was to determine the diagnostic accuracy of the PCR based assay against syndromic disease management (SDM) used in detection of vaginal discharge (VD) in symptomatic women. Sexually active women (21–56 years old) who complained of VD and visited OPD of Obstetrics & Gynecology during 2009 to Mid-2014 were recruited in the study. Based on clinical observations, patients were given treatment for either *Trichomonas vaginalis* (TV) or *Chlamydia trachomatis* (CT) or *Neisseria gonorrhoeae* (NG). Sample from each patient was also tested for presence of pathogen by PCR. Epidemiology, Prevalence, burden of multiple infection and overtreatment were calculated.1797 subjects were evaluated, 344 women (19.1%) had at least one infection while 1453 women (80.85%) tested negative for these three pathogens. Symptoms of sexually transmitted infection due to CT NG and TV are Itching, vaginitis, cervicitis, lower abdominal pain, dysuria, musty odor, irregular bleeding, Pain during intercourse and burning pain on maturation in women. Based on PCR diagnostic assay the prevalence of TV, NG and CT was 5.4%, 7.67% and 10.1% respectively. Additionally, 44 women (12.7%) had co-infection of CT + NG, 18 women (5.2%) had co-infection of CT + TV and 15 women (4.3%) were co-infected with NG + TV and 10 (2.9%) cases were co-infected with CT +NG +TV. Since all the patients (1797) were given antibiotics, the overtreatment rate was 80.85%. The infection for CT, TV and NG was more prevalent among women of reproductive age. SDM for Sexually transmitted infections using vaginal discharge as symptom are marred by high rate of over antibiotic treatment (80.85%). SDM alone is not an effective strategy for management of VD.

P212

In-silico Protocol Development for Substrate Identification for Point of Care Diagnosis of Acute Organophosphorus Poisoning

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Acetylcholinesterase (AChE) is the target biomarker for detection of acute organophosphorus (OP) poisoning. Its activity detection using acetylthiocholine, as substrate is currently the most popular method. However, acetylthiocholine detection method suffers from certain limitations to be used at point of care testing settings. Therefore, considerable current interest is generated for novel substrate identification for AChE that may be used for point of care diagnosis of OP poisoning. The objective was to develop in silico protocol for identification of substrates for detection of AChE activity at point of care. For virtual screening protocol development, the structures of the reported alternative substrates were downloaded from PubChem. The structures of AChE, BChE, albumin & esterase D were downloaded from Protein

Data Bank. Molecular docking was performed between the alternative substrates and the selected enzymes to understand the interactions. Enzyme substrate interactions were scored. To further validate the docking results, the enzyme substrate interactions were rescored by calculation of binding energy. Analysis of the docking and binding energy studies of different alternative substrates with AChE in comparison to other selected enzymes showed that certain substrates are more selective for AChE in terms of high fitness-score and favorable binding energy. So, the developed in silico protocol lead to the identification of candidate substrates for AChE activity determination.

P213

Random Urinary Protein-Creatinine Ratio – A Preadmission Test in Preeclampsia

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Pre-eclampsia is a leading cause of maternal and perinatal morbidity and mortality worldwide. It is a hypertensive disorder characterized by proteinuria, coagulation abnormalities and different systemic manifestations.The presence of proteinuria has both diagnostic and prognostic implications. Aim was to predict proteinuria during pregnancy, estimation of urinary protein to creatinine ratio is vital. The gold standard of for measuring proteinuria is 24 hrs urine collections, but a faster screening method is needed to save time. Spot urinary protein- creatinine ratio is however preferred for the purpose. Objective of this study was to evaluate the value of random urinary protein-creatinine ratio in prediction of 24 hr proteinuria in pre-eclampsia. The study included 30 preeclampsia cases and 30 normal healthy controls. Random urinary samples were collected and urinary protein (Pyrogallol method) and urinary creatinine (Jaffes method) were estimated. In our study, cases of preeclampsia showed high protein-creatinine ratios (with cutoff value of 0.25). In cases of preeclampsia, Protein-Creatinine ratios with 24 hours protein excretions showed an excellent correlation. Our study shows that protein-creatinine ratio in random urine sample is highly accurate for identification of significant proteinuria.We conclude that a random urinary protein-creatinine ratio predicts 24 hour urine protein excretion with high accuracy. It could be an alternative to 24 hour urine proteinuria as a preadmission test in pre eclamsia.

P214

To Establish the Reference Range of Glycated Hemoglobin

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Diabetes mellitus (DM) has emerged as a major healthcare problem in India. According to Diabetes Atlas published by the International Diabetes Federation (IDF), there were an estimated 40 million persons with DM in India in 2007 and this number is predicted to rise to almost 70 million people by 2025. The countries with the largest number of diabetic people will be India, China and USA by 2030. It is estimated that every fifth person with diabetes will be an Indian. Due to these sheer numbers, the economic burden due to DM in India is amongst the highest in the world.The aim was to establish the reference range for glycated hemoglobin (HbA_{1C}) in healthy non-diabetic subjects in our hospital laboratory and compare it with the values reported by standard laboratories.The study was conducted in the Department of Biochemistry, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana (Ambala, Haryana). Total number of subjects was 50 between the age of 30 to 70 years and divided into two groups: Group 1: 25 female subjects; Group 2: 25 male subjects. 2 ml of blood was collected from antecubital vein under aseptic conditions from each subject and put in EDTA vials. Hemolysed blood was estimated by semiautoanalyzer for HbA_1C. In females, the levels were 6.50 ± 0.74 % while in males the levels were 6.27 ± 0.94 %. The overall range in females was 4.8 - 7.56 % while in males it was 4.2 to 7.56 %. The values were comparable ($P>0.05$) with those reported by standard laboratories, e.g. Lal Path Lab (<6%), Charak Diagnosis (4.5-6.3%) and Mayo Clinic (6.5-7%). Our laboratory levels of HbA_{1C} are comparable with the reference range of different laboratories and hence suitable to be used as cut-offs while interpreting the results of patients with DM.

P215

Effect of Delayed Sample Processing & Storage of Serum in Respect of Glucose Estimation

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Usually significant time elapses between the collection and processing of the sample due to constraints of manpower and infrastructure, which leads to discrepancy in lab results. We quantify this discrepancy arising out of delayed sample processing in this

study. The aim was to study the effect of temperature and time on the glucose in serumand whole blood. Whole blood sample of 10 ml was collected from 32 subjects. Each sample was divided into four aliquots, 1staliquot of 4ml and three of 2ml each. The serum was separated using standard protocol. The serum glucose estimation was done by Glucose oxidase-peroxidase method coupled with Trinder's reaction on Erba XL 300 Biochemistry analyzer. The serum glucose of 1staliquot was labeled zero hour value. The remaining serum after 1st analysis was divided into three aliquots and stored at 2-8^{o}C. The stored serum was analyzed at 24 hr, 48 hr and 72 hr.The three aliquots of whole blood, stored at room temperature were processed & analyzed at 2hr, 4hr and 6 hr after collection. The whole blood sample stored at room temperature for 2hr, 4hr and 6 hr showed significant decrease ($P<0.001$) in glucose values compared to zero hour value. However, the glucose values remained statistically stable upto 72hrs on storage at 2 to 8^{o}C. Early serum sample separation is warranted to avoid decrease in true value of Glucose. Glucose levels remain statistically stable in serum, if stored at 2-8^{o} C upto 72 hours.

P216

Effect of Serum Storage Temperature and Time on Biochemical Parameters

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Laboratory tests are used by clinicians for diagnosis, monitoring and prognosis in patients with different diseases. A number of factors, pre analytical, analytical and normal biological variations affect the accuracy of the tests. The present study was designed to determine the effect of storage time and temperature on the laboratory results of 10 routine parameters in serum.The aim was to determine the effect of storage time and temperature on the laboratory results of 10 Biochemical parameter in the serum of healthy persons and to establish significance of timely transport and analysis of sample & its proper storage. This prospective analytical cross sectional study was conducted on 30 healthy persons in Medical college & S.S.G. Hospital, Baroda. In these samples we analysed 10 routine biochemical parameters. These samples were divided into 3 groups, Group 1, 2 & 3, 10 samples each. These samples were stored at room temperature, 4^{o}C and -20^{o}C respectively. In all these samples repeat analysis was done at 8, 24, 72 hours and results were compared statistically. At room temperature, Inorganic phosphate, Uric acid, Creatinine and Triglyceride level increased continuously, whereas Glucose and Bilirubin decreased. While at -20^{o}c most of the parameters were stable for 72 hours. Samples must be processed and analysed without delay. If delay in analysis is unavoidable, serum must be separated after centrifugation and refrigerated.

P217

Are Sex Hormones, Vitamins and Genetics Important Regulators of Bone Turnover in Men?

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Bone loss in ageing healthy men is multifactorial with hormonal, biochemical and genetic factors all being important. Objective was to elucidate the pathogenesis of age-related bone loss we studied some of the important biochemical, endocrine and genetic factors which directly or indirectly influence the bone health. 166 healthy men, 23-77 years with no history of fracture were recruited for this study. BMD was measured by DEXA. Blood samples were analyzed for Vitamin B12, Folic Acid, Homocysteine, Estradiol and Testosterone, and urine for Pyrilinks D, by chemiluminescence based immunoassays along with VDR gene polymorphism. Our results showed decrease in BMD, Pyrilinks D showed higher levels of upto 48.9% in 20 – 30 years group as compared to older ones. We observed an inverse relationship between, Homocysteine and Folate / Vitamin B12 levels. Our data showed minimal increase in Estradiol, significant decrease in Testosterone and Free Testosterone Index with advancing age. The ratio between Estradiol and Testosterone presumed to be an indirect index of aromatase activity showed significant increase with age. Two fold decrease in bioavailable Estradiol was observed, in 60 years and above age group as compared to 20 – 30 years. An increased frequency of the VDR Tt (44%) and TT (40%) genotype as compared to the wild type tt (20%) was observed. We, thus infer that sex hormones and vitamins, besides genetics are important regulators of bone turnover in men.The future inferential studies aimed at the prevention of osteoporosis should include monitoring endocrine and biochemical parameters along with risk alleles.

P218

Correlation of Glycemic Status with Various Anthropometric Parameters in Young North West Punjabi Population

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Overweight and obesity are recognized as recent threat which affects both developing and developed countries. Obesity and its associated morbidities are leading cause of most common non-communicable diseases. Anthropometric parameters such as BMI, waist circumference, waist hip ratio are useful indicators for

predicting Insulin resistance and Type 2 Diabetes Mellitus. The present study was planned with an objective of examining anthropometric parameters and glycemic status in offsprings of diabetics and to find a correlation if any between the two. A total of 742 young individuals were recruited for the study. These individuals were offsprings of diabetics and were siblings amongst themselves belonging to age group of 18-35 years. Various biochemical investigations such as fasting plasma glucose, Glycosylated Hb, S. insulin, C-peptide, apart from anthropometric measurements were carried out. Waist hip ratio and BMI had a positive highly significant correlation with fasting plasma glucose and HbA1c. Waist hip ratio had a negative significant correlation with β – cell function which was not true for BMI. Waist hip ratio was also significantly correlated with IR. Anthropometric parameters can be used to predict the glycemic status and loss of β-cell function of individuals. Comparison of waist hip ratio and BMI reveal that Waist hip ratio is a better indicator.

P219

Serum Activin B Levels as Predictive Biomarker for Ectopic Pregnancy

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Tubal Ectopic pregnancy remains to be a diagnostic dilemma with high morbidity and mortality. Identification of serum biomarkers for diagnosis of the above condition is warranted. Decidualization of endometrium is expected to be low in tubal Ectopic pregnancy due to limitation of space in the fallopian tube. Hence tubal ectopic pregnancy is likely to have less serum levels of decidualization markers and activin B is one such marker. In the present study, we explored the utility of activin B in discriminating tubal ectopic pregnancy from intrauterine miscarriages and normal viable intrauterine pregnancy. The study included 28 in tubal Ectopic pregnancy (tEP), 31 intrauterine miscarriages (IUM) and 29 normal intrauterine pregnancies (IUP) diagnosed by both ultrasonography and clinical examination. Serum activin B levels were measured at the time of admission. The median serum activin B levels were found to be significantly decreased in both tEP (P=0.004) and IUM (P=0.022) compared to normal IUP. When compared between tEP and IUM, activin B levels did not differ significantly. Receiver operating curve analysis demonstrated AUC of 0.722 and Youden's index of 0.4421 to discriminate ectopic pregnancy from viable IUP with levels less than 23.3 pg/ml delivering a sensitivity of 82.14%, specificity of 62.07%, and negative predictive value of 77.7% and positive predictive value of 68.4%. These results show serum activin B levels to be a promising biomarker in diagnosing ectopic pregnancy.

P220

Serum Heat Stable Alkaline Phosphatase in Hypertensive Disorder of Pregnancy

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It is generally recognized that a dependable test of placental function is one of the most urgent needs in obstetrics. The advantages of using various placental enzymes as an index of placental function were found in the fact that their measurements are simple procedures. The heat stable fraction of alkaline phosphatase is not destroyed when serum is heated at 65^{o}C for 30 min. It is secreted by placenta in first trimester and not present in healthy males and healthy non pregnant females. The aim was prediction of hypertensive disorder of pregnancy by assessing the level of HSAP and to establish its relationship with duration of pregnancy. Study was conducted on 100 women in their 2nd half of pregnancy who attended antenatal clinic in Department of Obstetrics and Gynecology in Regional Institute of Medical Sciences, Imphal, Manipur during Oct 2013 to April 2014. Serial estimations of Serum HSAP were done by the method of Kind and King. Levels of HSAP were found to be gradually increasing with the duration of pregnancy upto term. Level of HSAP showed a sudden high increase at about 15 days prior to the development of clinical detectable pre-eclamptic toxemia. It was also observed that level even if increased, falls again on improvement of condition by proper management**.** Estimation of HSAP is one of the easiest methods available. It showed increased levels in pre–eclamptic patients. Its detection in serum can help in early diagnosis and to adopt immediate measures to control it and to minimize maternal and fetal risk.

P221

Cystatin C- An Emerging Cardiovascular Risk Factor in Women with Polycystic Ovary Syndrome

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There is increasing evidence that patients with polycystic ovary syndrome (PCOS) have increased cardiovascular risk. This increased cardiovascular risk is probably the result of the metabolic disturbance associated with PCOS. Dyslipidemia, insulin resistance and obesity are all potent cardiovascular risk factors that tend to cluster in women with PCOS. Recent epidemiological studies have demonstrated the role of cystatin C as independent risk factor for

cardiovascular disease.The purpose of this study is to evaluate cystatin C levels in subjects with PCOS and to correlate with lipids and obesity. Study group comprised of 142 women with PCOS and 65 healthy non PCOS controls. Serum cystatin C, lipids, BMI and waist circumference were measured in PCOS subjects and age matched controls.The mean BMI, waist circumference, serum cholesterol, triglycerides, LDL-C and serum cystatin C values are significantly increased where as serum HDL -C was significantly decreased in PCOS subjects when compared with non PCOS controls. The present study has demonstrated increase in mean serum cystatin C levels in women with PCOS. Cystatin C correlated with lipids (cholesterol and LDL-C) and obesity.

P222

Relationship Between Body Iron Status and Cardiovascular Risk Factors in Patients with Coronary Artery Disease

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Iron is an essential trace element. It has a pivotal role in maintaining various cellular functions and enzyme reactions; whereas, iron overload has been known as a risk factor in progression of atherosclerosis. The aim of this study was to investigate the role of the serum iron, serum ferritin and total iron binding capacity (TIBC) in the causation of coronary artery disease (CAD). The study group consisted of 40 angiographically confirmed cases of CAD and 40 normal healthy controls taken from HIMS, Dehradun. The blood samples were taken after 10-12 hours of fasting. Serum lipids, serum lipoproteins, serum iron and TIBC were estimated by autoanalyzer (Dxc 900 Beckman Coulter). Serum ferritin was measured on Mini VIDAS and MDA was being done by Thiobarbituric acid method.The ratio of Total cholesterol and HDL was significantly raised in CAD patients (5.07) than controls (3.48). The levels of MDA were high in patients (19.9+1.7 µmol/l) as compared to controls (4.9 +1.0 µmol/l). Serum iron and Serum ferritin levels were significantly high in patients with CAD when compared with control groups (118.2+ 22.7mg/dl versus105+19.6 mg/dl, P<0.001) and (218.3+58.6mg/dl versus139.8+66 mg/dl, P<0.0001) respectively. TIBC levels were lower in patients than controls (309.8+79.2 versus 231.5+61.2, P<0.0001). This study concluded that increased levels of serum iron, ferritn and MDA might consider as risk factor for CAD in conjunction with other risk factors. The caution should be exercised in administration of iron supplements to patients of CAD.

P223

AOPP :A Novel Oxidative Stress Biomarker and its Relevance with Renal Failure Patients Undergoing Hemodialysis

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Hemodialysis is a life sustaining procedure for renal failure patients. More than 2,50,000 patients undergo hemodialysis globally. However, hemodialysis is associated with side effects that may be life threatening. Despite many advances in the case of patients on chronic maintenance hemodialysis over the past two decades, there has been virtually no change in the mortality of the dialyzing patients. Although patients on chronic hemodialysis have multiple comorbid conditions that predispose them to the development of infections, there is increasing evidence that enhanced oxidative stress is a major cause for mortality. Hence detection of the severity of the oxidant stress by a reliable biomarker is essential. Until recently biological evidence of oxidative stress in the dialysis patients in vivo relied entirely on measurement of lipid peroxidation byproducts such as MDA which in general poorly reflects the intensity of the oxidant stress. The exquisite vulnerability of proteins to reactive oxygen species is now well documented. The present review provides an insight into the role of a new potential biomarker advanced oxidative protein products (AOPP) in diagnosis and monitoring of oxidant stress in renal failure patients undergoing hemodialysis.

P224

Comparison of Lipid Accumulation Product Index: A Cheap Marker of Cardiovascular Risk with Body Mass Index, Body Adiposity Index and Waist Hip Ratio in Newly Diagnosed Polycystic Ovary Syndrome

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Metabolic derangements of polycystic ovary syndrome (PCOS) are contributing factors of increased the risk of cardiovascular disease. Lipid accumulation product (LAP) index as a cheap and reliable marker of cardiovascular risk and insulin resistance (IR) and metabolic syndrome (MS). The aim of this study was to estimate LAP index and compare with BMI, body adiposity index (BAI) and waist hip ratio (W/H ratio) in newly diagnosed PCOS patients

with controls. This is a case-control study including 30 PCOS patients aged between 15 and 40 years and 30 age matched healthy controls. Fasting sample were obtained for lipid profile analysis after getting written consent. Anthropometric measurements were taken as per protocol. LAP index, BMI, BAI and W/H ratio were calculated and analysed statistically. Among PCOS patients, LAP score had significantly positive correlation with patient's age (r = 0.42 & P=0.02), BMI (r = 0.441 & P=0.015), BAI (r = 0.45 & P=0.013) and W/H ratio (r = 0.405 & P=0.026). Among controls, LAP score had more significant positive correlation with W/H ratio (r = 0.546 & P=0.002). LAP score and W/H ratio had highly significant P value (P= 0.000) and BMI showed P= 0.003 while comparing PCOS patients and controls. LAP index, an easily obtainable, reliable, cheap marker of cardiovascular risk, IR and MS. The early detection and intervention could be possible to prevent metabolic and clinical complications among PCOS patients by LAP scoring.

P225

Development of Portable Mini Fluorescence Detector for Easy Visualization of Molecular Beacon Mediated Diagnostic Assay of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

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Culture method is commonly used for the screening of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). Introduction of Nucleic acid amplification test (NAAT) and Point of care testing (POCT) are widely accepted screening tests with high sensitivity and specificity for detection of CT and NG. However, the cost of the infrastructure and the expertise is too high to allow NAAT based assays for routine diagnosis and screening of CT and NG in developing countries like India. The aim of this study was to develop valid, reliable, affordable, cost effective mini fluorescent detector with ease of visualization. The main parts of this instrument are MicroLED fluorescence attachment with specific filter, monocular head, new design body (with no no sepiece), special objective and sample PCR tube holder. Beam of light passes from the sample containing PCR tube via the condenser which excite the flourophore. The fluorescent illuminated light passes from the 2.5X objective and the emission filter then visualized by the naked eye.The validation of detector was conducted by comparing the results for known positive specimen and known negative samples on the basis of fluorescent intensity obtained by ELISA reader and fluorescent detector. In both an instruments an excitation wavelength of 480nm, 550nm and emission wavelength of 520nm, 590nm was used for NG and CT respectively. The fluorescent detector gave high consistence results and was cost effective, easy to use, compact and portable instrument which can be used for detection of amplicon in any molecular beacon based assay.

P226

Level of Serum Myeloperoxidase(MPO) in Different Stages of Chronic Kidney Disease (CKD)

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Many authors have demonstrated the presence of MPO containing cells as well as MPO proteins and its activity in renal diseases.There are evidences that suggest that patient suffering from CKD are at increased risk for CVD. The aim was to study serum myeloperoxidese level in different stages of chronic kidney disease. Serum MPO was estimated by sandwich ELISA method in 30 controls and 30 cases. Cases were categorized into 5 stages based on their GFR calculated by Gault Cockroft formula. The statistical analysis for baseline characteristics and serum MPO levels between cases and controls was done by using parametric test (unpaired't'test) for quantitative analysis. Between-group comparisons were performed using 1- way analysis of variance (ANOVA). On comparison of the level of serum MPO (values in Mean±SD) between the cases (18.661±13.088) and controls (61.761±24.840) highly significant difference was noted (P-value =0.01). A highly significant difference was also found on comparison of the levels of serum MPO in various stages of CKD [Stage1 (n=6) 103.422±7.043], [Stage2 (n=6) 26.862±2.136], [Stage3 (n=6) 14.698±3.796], [Stage4 (n=6) 9.325±1.717] and [Stage5 (n=6) 4.068±1.302] in the cases(P-value =0.01). There was rapid decline in serum MPO level with advancing chronic renal failure which appeared to be caused by a synergism of different mechanism such as inflammation, oxidative stress and genetic components.

P227

SAAG in Differential Diagnosis of Ascites

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Ascites is one of the most common clinical problems confronting a physician and ascitic fluid analysis is the most effective way to diagnose it. The classification of ascites into 'transudative' and

'exudative' has lately been challenged; it offers little insight to the pathophysiology of ascitic fluid formation. These drawbacks led to new approach to classify ascites, based on albumin gradient between serum and ascitic fluid. The aim was to differentiate ascites on the basis of SAAG and compare the diagnostic accuracy of SAAG with that of ascitic fluid total protein (aTP). A cross-sectional study on 100 patients with ascites, admitted in Medicine ward, Regional Institute of Medical Sciences, Imphal, Manipur, conducted during the period of August 2013 to August 2014. The patients were subgroup into ascites with portal hypertension (n=60) and without portal hypertension (n=40). Estimation of albumin in ascitic fluid and serum and total protein were estimated with established colorimetric methods.The aTP values in Group A and B had a mean of 2.22 ± 0.63 gm/dl (P< 0.001) and 3.34 ± 0.7 gm/dl (P<0.001) respectively. The SAAG in Group A and B had a mean of 1.81 ± 0.49 gm/dl (P<0.001) and 0.75±0.27 gm/dl (P<0.001) respectively. SAAG and aTP had a sensitivity of 95% and 61.66% and a diagnostic accuracy of 95% and 68% respectively in the differential diagnosis of ascites. SAAG a better biochemical parameter in classifying ascitic fluid collection of varied aetiology than aTP.

P228

Assessment of Glycaemic Status in Cerebrovascular Accident Patients and its Relation to Clinical Outcome

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Cerebrovascular accidents (CVA) or stroke is one of the leading causes of mortality and morbidity worldwide. There is increasedrisk and prevalence of stroke in diabetic patients. Diabetics as well as patients with stress hyperglycaemia have severe stroke and these patients are associated with poor prognosis.The aim was toassess the glycaemic status in CVA patients and to find out its relation to clinical outcome.The study was conducted among 50 cases of acute stroke admitted in Medicine ward RIMS, Imphal during the period of November 2013 to August 2014. Clinical records of patients, estimation of the plasma venous glucose level and glycosylated haemoglobin (HbA1C) by colorimetric and ELISA method respectively were performed. Criteria for diagnosis of diabetes mellitus were done following American Diabetes Association (ADA, 2011). 20 cases of stroke were euglycaemic (random plasma glucose <200mg% and HbA_{1C}<6.5%), 30 cases were hyperglycaemic (random plasma glucose >200mg %). Out of those patients with hyperglycaemia, 14 cases (28%) occurred in known diabetes patients (patients with history of diabetes & HbA_{1C}>6.5%), 10 cases (20%) occurred in new diabetes patients (no history of diabetes but HbA_{1C}>6.5%), and 6 cases (12%) was in stress hyperglycaemia (HbA_{1C}<6.5%). Poor clinical outcome was found in stress hyperglycaemics (66.67% mortality), known diabetes (28.6% mortality) and newly detected diabetes (20% mortality). Severity of stroke correlates with the glycaemic status of the patients in diabetics and non-diabetics. Hyperglycaemia in non-diabetic patients after acute stroke is a stress response reflecting more severe neurological damage.

P229

Study of Cardiac Markers in Myocardial Infarction

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Myocardial infarction is a global health problem with increasing incidence world wide. It is the first leading cause of morbidity and mortality. About 2.3 million patients die with IHD per year.The present study was conducted on 50 patients in which about 40% patients had MI with non diagnostic ECGs and included history of hypertension (8), history of DM (15) history of smoking (21), history of alcohol intake (9) history of tobacco (5) history of chest pain (45) non ST segment elevation MI (20) ST segment elevation MI (30) (AWMI – 20 IWMI- 5 ASWMI – 3 , Post wall MI – 2). Their blood samples were collected and were analysed for LDH , SGOT, CK-MB & Trop – T. The result of these patients were compared with the normal healthy controls. The SGOT , LDH , CK-MB & Trop- T were significantly increased in the MI patients with non diagnostic ECGs (P<0.001) therefore this study shows these biochemical parameters are better indicators of MI as compared to non diagnostic ECGs.

P230

Cystatin C in Severity of Coronary Artery Disease

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Cystatin C is a 13 KD protein composed of 120 amino acid residues which produce in all nucleate cells of the body. Cystatin C was a recognized marker of renal dysfunction, is gaining importance in dysfunction of other organs as well. Preliminary studies indicated a role for cystatin C as a projecting marker in coronary artery disease (CAD). CAD is the primary cause of death and has emerged as major health burden worldwide. The prevalence is increasing as well and is affecting younger age group. By 2020, 60% of the world's heart disease is expected to arise in India. The fundamental cause of CAD is atherosclerosis.There is a proof that

both elastolytic cysteine proteases (cathepsins) and their inhibitors, an important one being cystatin C are implicated in the pathogenesis of atherosclerosis.The aim was to assess the of serum cystatin C levels in CAD and its clinical spectrum. The spectrum of CAD is Stable angina (SA), unstable angina (UA) and Myocardial infarction (MI). Study group comprised of 135 patients diagnosed as having CAD based on clinical and bio-chemical criteria. Control group included 56 age and sex matched subjects (non CAD cases) using the above mentioned criteria. In this study significant enhance of mean serum cystatin C levels were observed in CAD cases when compared with controls. Highest mean cystatin C values were observed in MI than UA and SA. Serum cystatin C plays a significant role in the progress of CAD and it might have a role as a prognostic marker of CAD.

P231

Genotyping of ABCA1, CETP and APOA1 Gene Variants in Coronary Artery Disease Subjects

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Plasma measures of lipids are well established risk factors for Coronary Artery Disease (CAD). A low level of serum HDL-C is an independent risk factor for CAD. Blood lipids are complex genetic phenotypes, influenced by both environmental and genetic factors.The objective was to test the SNP's of ABCA1, CETP and APOA1 genes and to associate the variants with CAD.150 angiographically verified CAD patients with 150 angiographically negative, age and sex matched CAD controls, visiting the P.D. Hinduja Hospital and Medical Research Center's catheterization laboratory were recruited for the study.The ABCA1 variants E1172D (rs33918808), I883M (rs2066718), V771M (rs2066714) and R219K (rs2234884), V825I (rs2066715), T1427 (rs2066716) were genotyped by Multiplex Allele Specific PCR. CETP Variant Taq1B (rs 708272) was genotyped by Tetra ARMS PCR and ApoA1 variant -75 G>A (rs no 670) was genotyped by PCR-RFLP. Frequency of the variants of I883M (controls-24.2%, cases-19.3%), V771M (controls- 12.1%, cases- 10.0%) and V825I (controls-8.1%, cases-6.7%) were observed to be higher in controls over cases. T1427 variant (cases - 8.7%, controls - 4.0%) was seen in more cases than controls. Higher frequency of the ApoA1 variant was seen in the subjects genotyped, but was similar in both cases and controls (37%). The frequency of CETP Taq1B variant was more in cases (31.1%) than in controls (25.7%).This is an ongoing study and preliminary results suggest that CETP Taq1B variant may be associated with CAD.

P232

Role of Platelets in the Pathogenesis of Thrombosis in Patients with Antiphospholipid Syndrome

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Pathogenesis of APS is an ongoing area of research and studying the role of platelets will be helpful in developing newer diagnostic and therapeutic strategies.The objective was to delineate the role of platelets in thrombotic process in patients with antiphospholipid syndrome (APS). Forty patients with APS, diagnosed as per modified 2006 Sapporo's Criteria and who were not on aspirin or any other antiplatelet drug, were included. The same number of age- and sex-matched healthy controls was also recruited for comparison. The following platelet function studies were performed using the blood samples collected from APS patients as well as healthy controls: platelet aggregation studies, platelet secretion of dense granules (a. total degranulation b. platelet secretion of granules in relation to time c. visualization of platelet degranulation), clot retraction studies, and western blot studies on clot retracted samples for demonstration of activated proteomes. A significant increase ($P < 0.001$) in the platelet aggregation in APS patients as compared to healthy controls was noted. The subjects also showed a significant increase ($P < 0.05$) in the platelet granule release as well as more degranulation ($P < 0.001$) in relation to time at stored condition, which were well-visualized under phase-contrast microscope. Sixty-five percent of APS patients showed lesser as well as delayed clot retraction as compared to healthy controls, signifying that the platelet clots are less retractile in APS patients.The study clearly demonstrates the hyperactivity of platelets in APS patients in each step of their activation as compared to the controls. This indicates the major role played by platelets in APS pathogenesis.

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Cystatin C as Marker in Myocardial Infarction

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Cystatin-C, a cysteine protease inhibitor is a new and better endogenous marker for renal dysfunction than creatinine.

However its role in patients of myocardial infarction remains less researched. The objective was to establish role of Cystatin C as a marker in myocardial infarction. This was a cross sectional study carried out at Pt.B.D.Sharma PGIMS, Rohtak. Blood samples of forty males (Troponin-T positive) known to have myocardial infarction and thirty males (controls, Troponin-T negative) were collected after informed verbal consent. Serum marker cystatin C was measured using particle enhanced immunoturbidimetry method by kit from Accurax. The mean level of serum cystatin C (2.46 ± 0.33 mg/L) in patients with myocardial infarction when compared to controls. Increased serum cystatin C was an independent predictor of all-cause mortality and combined events (all-cause mortality and MI) after adjustment to non-biomarker baseline factors. Increased levels of serum Cystatin C were found in patients with acute myocardial infarction without established chronic kidney disease.

P234

Correlation of Serum Beta$_2$-Microglobulin with Serum Creatinine and Estimated Glomerular Filtration Rate in Patients with Early Stages of Chronic Kidney Disease

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Chronic kidney disease (CKD) is a global health problem with rising incidence. Serum creatinine (SCr) is insensitive to moderate reductions in glomerular filtration rate (GFR). Low molecular weight proteins like beta$_2$-microglobulin (BMG) are cleared by the plasma through glomerular filtration. Hence serum concentrations increase progressively with reduction of GFR.The objective of the study was to correlate serum concentrations of BMG with creatinine and estimated GFR (eGFR) in patients with early stages of CKD. 74 adults in early stages of CKD were included based on eGFR, calculated using the 4 variable MDRD (Modification of Diet in Renal Disease) equation and albumin creatinine ratio. They were divided into four groups based on the stages of CKD. SCr was measured using Jaffes reaction with Rosche Hitachi P800 autoanalyser and serum BMG was measured using Calbiotech ELISA kit and compared using one way ANOVA and Pearson's correlation tests with SPSS version 16 software. Levels of serum BMG were significantly elevated in all groups, ($P<0.01$) while SCr levels were in normal range in patients with eGFR >60ml/min/1.73m^2. Both BMG (r=-0.792) and SCr (r=-0.913) increased with reduction of eGFR ($P<0.01$). Correlation with eGFR in stage1 CKD showed serum BMG (r=-0.824, $P<0.01$) and SCr (r=-0.362) and in stage2 CKD, BMG (r=-0.705 $P<0.01$) and SCr (r=-0.609, $P<0.01$). Serum BMG is elevated in asymptomatic patients with normal creatinine, thereby demonstrating its reliability in detecting early stages of CKD. Serum BMG may possibly substitute creatinine in diagnosing early stages of CKD and facilitate appropriate therapeutic interventions.

P235

Role of Serum Calcium, Sodium & Potassium in Patients with Essential Hypertension

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Hypertension is a common and complex human disease.The mineral elements namely Sodium, Potassium and Calcium have a central role in regulating blood pressure influencing both the peripheral ressistance and cardiac output. The objective was to measure the serum calcium,sodium and potassium level and their role in patients with Essential Hypertension and to find out Case control study was conducted among 50 patients with Essential Hypertension attending outpatient department of Cardiology of RIMS hospital, Imphal and 50 healthy subjects were taken as controls during the period of December 2013 to September 2014. Estimation of serum calcium was done by spectrophotometric measurement. Estimation of serum sodium and potassium were done by Flame emission photometry. Blood glucose, serum albumin, serum creatinine, blood urea, total cholesterol, complete blood count was done by standard laboratory techniques. Results depicted that:- The serum calcium level in test group was mean value of 8.38mg/dl (7.2-9.2) whereas in control group 9.3mg/dl (8.3-10.1) in which test group had showed significantly low values ($P<0.001$). The serum sodium level in test group was mean value of 146.30 mmol/l whereas control group showed mean value of 138.68 mmol/l. So, which is statiscally significant. The serum potassium had a mean value of 4.22 mmol/l in test group and mean value of 4.18 mmol/l in control group which was statistically insignificant ($P>0.05$). Present study shows that high intake of sodium and low intake of potassium, calcium maintain elevated blood pressure. So, for the prevention and basic treatment of elevated blood pressure various methods to decrease intake of sodium and increase intake of potassium, calcium should be comprehensively applied.

P236

Association of Angiotensinogen Converting Enzyme Gene Insertion / Deletion Polymorphism with Risk of Ischemic Heart Disease in Smokers from South India

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Smoking is a major risk factor for ischemic heart disease (IHD). The deletion (D) allele of the angiotensinogen converting enzyme (ACE) gene polymorphism has been associated with hypertension, ischemic stoke and myocardial infarction. The present study was carried out to determine the association of the ACE gene insertion/deletion (I/D) polymorphism and myocardial contractility with smoking in IHD patients.107 Male IHD patients admitted consecutively in the cardiology unit of a Government hospital and 100 age and sex matched healthy controls were enrolled in this study.The patients were further divided into smokers and nonsmokers. All the subjects were genotyped for ACE I/D polymorphism by polymerase chain reaction (PCR) and investigated for dyslipedemia and myocardial ejection fraction. We found significant difference in the distribution of D allele between patients and controls (OR 1.69, 95%CI 1.139 to 2.517, P=0.009).The significantly lower EF (P<0.001) was suggestive of greater cardiovascular compromise in smokers. The frequency of ID genotype was significantly associated with cases compared to controls (OR 2.054, 95% CI .1694-3.624 P=0.012) but was not significantly associated with smokers as compared to non smokers. Multiple dimensionality reduction analysis (MDR) indicated that ACE-D allele confer 3.76% to IHD, while TC & LDL contribute 75& 60% respectively.We infer significant association of D allele with IHD. This is the first time using MDR biomarkers have been selected to identify smokers at risk of developing IHD.

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Study of Methylene Tetra Hydro Folate Reductase [MTHFR] Gene Polymorphism in Stroke

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Blockage of small arteries in the brain causes serious disturbance of brain functions a condition called as stoke. Deficiency of MTHFR that causes impairment of the methionine metabolism leads to hyper homocysteinemia. This variant has become recognized as the most common genetic cause of MTHFR gene polymorphism. The aim was to study the mutation of MTHFR gene polymorphism in stroke patients and homocysteine levels and NO synthase levels in stroke patients. To study the oxidative stress and antioxidants mechanism in the stroke patients, 30 stroke patients with the help of neurology department were recruited as cases. Age matched normal healthy 30 were controls who were staff and students of Narayana Medical College, Nellore. MTHFR gene plymorphism studied by PCR and gel electrophorosis. Nitric Oxide synthase estimated by kinetic method.The results indicated prolonged vasoconstriction and increased oxidative stress. The gene polymorphism of MTHFR is one of the risk factor for the stroke. C677 T the MTHFR gene located on 1P $^{36.}$ This study of MTHFR gene polymorphism might allow us to know the role of gene therapy for stroke patients.

P238

Association of Lipoprotein-Associated Phospholipase A_2 Activity and Urinary F2-Isoprostane in Diabetic Patients with and without Nephropathy

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Oxidative stress is a major factor contributing towards diabetic complications such as diabetic kidney disease. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a lipoprotein-bound enzyme that catalyzes the hydrolysis of oxidized phospholipidsliberating arachidonic acid, which on peroxidation leads to the formation of F2-isoprostane which is a biomarker of lipid peroxidation.The aim was to evaluate theactivity ofplasma Lp-PLA2 and levels of urinary F2-isoprostanes and

malondialdehyde (MDA) and to correlate them in diabetic patients with and without nephropathy.This study comprised of 90 participants viz: 30 healthy controls (HC), 30 Type 2 diabetic patients (T2DM) and 30 Type 2 diabetic patients with nephropathy (DM-CKD). Plasma Lp-PLA_2 activity and malondialdehyde (MDA) were measured by colorimetric assay and urinary isoprostane levels were quantified by ELISA. Highest plasma Lp-PLA_2 activity (39.64±9.01, P<0.05) was observed in DM-CKD patients which was significantly higher as compared to HC. Similar trend wasalso observed for F2-isoprostane (105.21±40.23, P<0.05) and MDA (5.84±1.16; P<0.05) in DM-CKD vs HC. Lp-PLA_2 activity and Isoprostane levels were found to be positively correlated in patient groups; MDA and Isoprostane levels were also found to bepositively correlated in patient groups but results were not statistically significant. These results illustrate that elevated activity of Lp-PLA_2 in DM leads to increased lipid peroxidation as suggested by increased Isoprostane and MDA levels. Therefore, patients with high Lp-PLA_2activity have higher levels of oxidative stress leading to oxidative damage, particularly in kidney.

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Lipid Profile and Serum Ferritin Level in Indian Patients of Acute Myocardial Infarction

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National Health and Nutrition Examination Survey (NHANES III), 1988-1994, first time reported a significant, positive association in iron storage and heart disease risk. Thereafter several researchers have found an association between iron overload, SF and MI. No such Indian study was available in the literature and so we decided to find out a correlation between SF level and AMI in Indian patients. Fifty Indian patients of AMI (study group) and fifty Indian healthy volunteers (control group) were included for the present study. Lipid profile including TC, HDL-C, LDL-C, VLDL-C& TG and Serum ferritin levels were estimated in all subjects by literature approved methods. Mean ± SD of TC level was 250.64 ± 25.61, of HDL-C was 36.52 ± 2.86, of LDL-C was 165.69 ± 26.80, of VLDL-C was 42.35 ± 8.53 and of TG was 211.83 ± 42.65 in study group while these values were 174.46±47.68, 43.2±12.52, 98.37±41.13, 32.88±21.45, 164.42±107.29 respectively in control group. All the parameters were found not only raised in patients of AMI but were also statistically significant when compared with control group (P=<0.01). Mean ± SD of serum ferritin levels was 268.43±30.17 ng/ml in study group and 110.96±56.5 ng/ml in control group; this level was found not only raised in patients of AMI but were also statistically significant when compared with control group (P=<0.01). We concluded that significantly increased level of all five parameters of lipid profile and SF werefound in Indian patients of AMI.

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Association of Plasma Malondialdehyde (MDA), Whole Blood Glutathione Peroxidase (GPx) in Pulmonary Tuberculosis

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TB kills one person every 90 seconds in India and about 1000 people every day. M. tuberculosis replicates in the host macrophages and results in free radical burst which induce lipid peroxidation of PUFA and forms MDA. MDA is responsible for some of the damaging effects of free radicals on Deoxyribonucleic acid and on cell membranes. It can be measured in blood as a parameter of oxidative stress and so the present study was conducted to find out if there exists an association of plasma malondialdehyde and glutathione peroxidase in pulmonary tuberculosis (PTB). The aim was to explore any association of MDA and GPx levels in PTB patients. 100 patients were identified from the respiratory medicine Department of Chhatrapati Shivaji Subharti Hospital, Meerut and were enrolled for the present study after ethical clearance. Informed consent was taken from each patient. An age, sex and number matched control group was also taken. After confirming pulmonary tuberculosis in all these patients; MDA was measuredby OxiSelect™ TBARS Assay Kit; and GPx by using the method described by Paglia and Valentine spectrophotometrically. The data so collected was analysed using Mean, Standard deviation, Students (t) test and Pearson correlation coefficient (r) (P value <0.05 &<0.01 was considered to be statistically significant. In our study the values of MDA was found significantly higher in study group when compared with control (P value <0.01); GPx was found significantly lower in study group when compared with control (P value <0.01). The Plasma Malondialdehyde is significantly higher and Glutathione peroxidase is significantly lower in patients of Pulmonary tuberculosis when compared with healthy control.

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Relationship of Serum Lactate Dehydrogenase and Alkaline Phosphatase with Lung Cancer

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Enzymes can be used as diagnostic and prognostic markers in lung cancer patients for detection, monitoring and evaluation of treatment.The objective of the study was to analyze the level of serum Lactate dehydrogenase (LDH), and Alkaline phosphatase (ALP) activity in lung cancer patients receiving treatment. A total of 50 lung cancer patients suffering from different stages of the disease were selected for the study. All patients were freshly diagnosed and clinically staged. A total of 30 age, sex matched apparently healthy individuals were taken as the "control group." Study result reveals that mean serum LDH (439.42±145.31 IU/l) and ALP (299.60±144.29 U/l) are significantly higher in patients with lung cancer as compared with control group (LDH 221.06±34.00 IU/l and ALP 162.30±20.61U/l). The difference is statistically significant. Serum levels of LDH and ALP are also seen to rise with stages of disease showing positive correlation. The serum levels of LDH and ALP are also seen to reduce in response to therapy with tumor mass regression, which was more pronounced with serum LDH. Measurement of serum LDH and ALP activity can be useful marker in lung cancer diagnosis, in monitoring disease progression and evaluating response to therapy.

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Common Variants of *SLCO1B1, CYP3A4* and *CYP7A1*Genes as Predictors of Lipid-Lowering Response to Atorvastatin Therapy

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Statins are widely prescribed and are established as first-line therapy for the primaryand secondary prevention of coronary artery disease (CAD). However, there is large inter-individual variability in clinical response to statin treatment. Several studies have reported genetic variation contributes to variable reduction in low-density lipoprotein-cholesterol (LDL-C) levels in response to atorvastatin therapy. Genetic variations in genes involved in statin and lipid metabolism are proposed as important determinants of statin response.This study evaluated the association between known variations of *SLCO1B1, CYP3A4* and *CYP7A1* genes and atorvastatin therapy. Genotypes were determined by using multiplexallele specific-polymerase chain reaction (AS-PCR) in 100 hypercholesterolemic patients, treated with 10mg of atorvastatin for 8 weeks. Baseline & after 8 weeks LDL-C levels were determined for subjects.The genotype distribution for all polymorphisms investigated was consistent with Hardy–Weinberg equilibrium. Our results show that, the variant allele (C) for *CYP7A1*(rs3808607) polymorphism is associated with -26±1.8% LDL-C reduction, while wild type allele (A) showing -32±4% reduction in LDL-C.We also see that, for *CYP3A4* promoter variant (rs2740574), individuals with AA genotype exhibited a greater reduction in LDL-C as compared to AG+GG genotype (-30 ±1% Vs.-27±1.3%). However we did not observe any difference in LDL-C reduction for *CYP3A4* missense variant (rs4986910). We found for both the polymorphisms of *SLCO1B1* (rs2306283, rs11045819) variant allele showing higher reduction in LDL-C as compared to wild type. These results suggest that polymorphisms in lipid and statin pathway genes are associated with variable reduction in LDL-C. Hence inclusion of pharmacogenetic data along with clinical parameters would assist in atorvastatin dosage.

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Study on Serum Lipid Profile, Homocysteine, Lipoprotein(a) and Apolipoprotein (A1) in Pre-eclampsia

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Elevated serum homocysteine, Lipoprotein(a) are risk factors for endothelial dysfunction and vascular disease while Apolipoprotein (A1) reduces it. We compared serum homocysteine, lipoprotein (a) and Apolipoprotein (A1) levels in healthy pregnant women and preeclampsia and investigate the relationship between these parameters. A Cross sectional study was conducted with collaboration of Dept. of Gynae & Obs. and Biochemistry on 66 pregnant women in M.G. M. Medical college hospital kishanganj, Bihar. 66 pregnant women participated in the study of whom 33 were pre-eclamptic and 33 were healthy pregnant women. 32 (96.96%) of cases and 30 (90.9%) of controls are having lower (<30 mg/dl) Lp(a) levels and only 1 (3.03%) of cases and 3 (9.09%) of controls have higher range (> 30mg/dl). 33 (100%) of cases and 32 (96.96%) of control has normal range of homocysteine (<15 mmol/l). 32 (96.96%) of controls and 33 (100%) of cases are of higher range of APO (A1) Levels (>120 mg/dl).

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Serum Homocysteine Levels in Osteosarcoma

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Hyperhomocysteinemia has been associated with atherosclerosis, preeclampsia, neural tube defects and cancers. Elevated homocysteine has been suggested as an independent risk factor for cancer. The present study was planned to analyze the status of serum homocysteine in patients with osteosarcoma. The present study was conducted in sixty orthopaedics patients who were divided into two groups, Group I with thirty patients of osteosarcoma and Group II with thirty patients with musculoskeletal pain. Five ml venous samples were drawn before starting any treatment (which is mainly amputation of affected limb) and serum was separated by centrifugation. Serum homocysteine was estimated by competitive immunoassay using direct chemiluminiscence technology and data so obtained was subjected to SPSS version 1.8 and student's t-test and regression analysis was carried out. The present study indicated that serum homocysteine levels were increased in osteosarcoma patients as compared to controls. Raised homocysteine levels could be due to contribution from rapidly proliferating cells. The present study suggests that homocysteine can serve as useful markers for diagnosis and follow up of disease.

P245

Clinical Significance of Tumor Markers in Hepatocellular Carcinoma

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Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer cells can spread to other parts of the body through the blood and lymph systems. The main categories of cancer include Carcinoma, Sarcoma, Leukemia, Lymphoma and myeloma, Central nervous system cancers. Hepatocellular carcinoma (HCC) is the part of gastrointestinal tract cancer (GIT) as the gastrointestinal tract (GIT) is the site of more cancers than any other organ system in the body. Hepatocellular carcinoma (HCC) is one of the most common malignant tumors occurring in males in the world. The annual incidence of the disease worldwide is estimated to be 1,000,000 cases, with a male to female ratio of about 4:1. Primary hepatocellular carcinoma (HCC) is an important cause of death in patients with chronic liver disease and in carriers of hepatitis B and C virus. 156 patients selected for the present study were outdoor/ indoor patients from Tata Memorial Hospital Mumbai. An in house diagnosis of the patients with Hepatocellular carcinoma was established on the basis of detailed clinical history, clinical examinations, Histopathology reports, liver scan, CT scan, FNAC and other relevant laboratory investigations.The serum levels of AFP and CEA and CA19-9 and CA242 significantly increased proportionately whereas the serum levels of AFP and CA19-9, AFP and CA242, CEA and CA19-9, CEA and CA242 were statistically not significant. All the specified co-related makers were inversely proportional to each other. Therefore AFP, CEA, CA19-9 and CA242 makers play significantly an important role in detecting HCC patients in all stages.

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Evaluation of Salivary and Serum Tumor Markers in Breast Cancer Patients

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Saliva is an important and necessary body fluid. In the last 10 years the use of saliva as a diagnostic fluid has become somewhat of a translational research story. Based on this, a study has been carried out in our department to evaluate the salivary and serum tumor marker level in breast cancer patients. Unstimulated saliva and blood samples were collected from the histopathologically diagnosed breast cancer patients from our OPD after getting institutional ethical clearance and before initiation of any treatment in fasting condition. The study group comprised of 100 patients and control group comprised of 25 healthy normal individuals in the same age group from our hospital staff and volunteers. Blood samples were centrifuged at 2000 rpm for 10 minutes. The collected saliva was also centrifuged within one hour to eliminate debris and cellular materials. All the marker (CEA, CA 15-3, and CA 125) levels in both samples were measured on the same day by using Vitros Eci (Ortho clinical Diagnostics Ltd) CLIA instrument as per manufacturer's procedures. The values were analyzed using SPSS statistical software and expressed as mean + SD, the levels of significance were determined by Student's 't' test. In our study we found that there was a significant positive correlation between Salivary and serum concentration of these markers. Salivary analysis is advantageous due to the easy and non invasive method of collection, safety and the possibility of repeated collection without discomfort to the patients. So tumor marker analysis of saliva can be used (an alternate to blood) to diagnose and monitoring treatment procedure and disease recurrence in breast cancer patients.

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Quercetin Pentaacetate and 7,8-Diacetoxy-4-Methyl Coumarininduced Apoptosis in Lung Cancer

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Lung cancer has been the most common cancer in the world for several decades.Worldwide 1.61 million new cases of lung cancer are registered every year, representing 12.7% of all the new cancers. Quercetin Pentaacetate (QPA) and 7,8-Diacetoxy-4-Methyl Coumarin (DAMC) are the heterocyclic polyphenolic acetates that possess a wide array of biological functions including anti-inflammation, anti-carcinogenesis, anti-oxidation and anti-proliferative in a number of *in vitro* and *in vivo* models. We have analyzed the effects of various polyphenolic acetates on lung cancer. The present study was undertaken with the aim: To study apoptosis and cell cycle arrest in QPA and DAMC treated Non small cell lung carcinoma cells (A549 cells). Non small cell lung cancer cell line (A549) is treated with various Polyphenolic acetates (QPA* and DAMC**). Apoptosis is analyzed by studying the morphological features of cells using fluorescent microscope after staining the cells with DAPI. Apoptosis and Cell cycle arrest are further analyzed by the appearance of hypo-diploid (sub G1) population using RNase and PI and Annexin- V FITC kit by Flow-cytometer. The data is presented as the Mean ± S.E.M and statistically significant differences among groups is assessed by using analysis of variance (ANOVA) followed by Post hoc test. 'P' value of = 0.05 is considered statistically significant. Polyphenolic acetates (QPA and DAMC) have been found to significantly increase cell cycle arrest and apoptosis in A549 cells. So, they qualify as the potential cancer chemotherapeutic or chemopreventive agents for the treatment of NSCLC cells. However, further studies are required to determine their therapeutic efficacies in treatment of NSCLC.

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Serum Vitamin D and Parathyroid Hormone Levels in Chronic Kidney Disease Patients on Haemodialysis

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Chronic kidney disease (CKD) is associated with biochemical and clinical abnormalities, including chronic kidney disease-mineral and bone disorder (CKD-MBD). Despite recent therapeutic advances, mortality remains high among patients with chronic kidney disease maintained on haemodialysis (HD). Hence, the clinician should evaluate trends in biochemical markers as a part of an integrated and comprehensive assessment and to guide the treatment. Therefore it is strongly recommended to monitor serum levels of calcium, phosphorus, Vit D and PTH in chronic kidney disease patients. A case control study was done in Department of Biochemistry &Department of Medicine, VMMC and SJH, New Delhi. Serum Vitamin D and Parathyroid hormone levels were measured by Enzyme linked immunosorbent assay (ELISA). The data were expressed as mean± SD. The mean serum Vit D level was found to be 41.09 ng/ml in control subjects and 21.6 ng/ml in cases (patients with CKD stage 4-5 on haemodialysis). Mean serum PTH hormone level was found to be 32.23μg/ml in controls and 314.9μg/ml in cases. Serum Vit D level was significantly ($P<0.005$) low and PTH hormone levels were significantly ($P<0.005$) high in cases as compared to controls. The results of this study not only confirm the previously reported associations between Vit D, PTH hormone levels in CKD patients but they also suggest that these levels may have more long-lasting implications. Severe secondary hyperparathyroidism is associated with morbidity and mortality in patients with CKD. So, Vitamin D supplementation may prove as recommended modality for prevention and treatment of hyperparathyroidism in patients on dialysis.

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Gene as an Internal Control for the Quality Assessment of Polymerase Chain Reaction Methods for the Diagnosis of Infectious & Autoimmune Diseases

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Molecular diagnostics club all the techniquesto analyze biological markers and gene variations in DNA sequences in the genome and proteome and how their cells express their genes as proteins by applying molecular biology to medical testing? Evaluation and interpretations of the observations in molecular diagnosis plays a vital role in patients care, disease management as well in clinical research. The usage of genes for various controls: positive, negative and internal are very significant for concluding the diagnostics efficacy and sensitivity. The current study was planned to evaluate the different specimens coming for molecular diagnosis about the different internal controls and the disorders in which they can be utilized. A total of 50 specimens from different departments of SMI Hospital, SGRRIM&HS, Dehradun were collected. Specimens includes: cervical swabs for Human Papilloma Virus (HPV) genotyping, blood for HLA-B27, and CSF for Herpes Virus (HSV) genotyping and Tuberculous Meningitis (TBM). Theywere processed with variants of PCR for molecular Characterization. α-keratin gene was used as an internal control for HSV genotyping & Nested TB PCR evaluation, β-actin gene for Cervical specimensin HPV genotyping and Human growth hormone (HGH) and β globulin genes for sequence specific allele PCR for HLA-B27. Two cases for Nested PCR for Tuberculosis came invalid and were reevaluated for the proper PCR results. Validation of the PCR protocols was standardized for most of the molecular diagnostics assay procedures. Quality assessment and quantification of the source specimens for the molecular diagnosis can be verified by the usage of the various housekeeping genes and other DNA sequences. A true internal control provides assurance about the collection, transportation and amplification of the target analyte, right from the patient to result analysis. Using housekeeping genes/DNA sequences as an internal control in molecular based assay techniques may be helpful for the corrections and checking the pre-analytical errors in the specimen collection procedures and will help to prevent false negative results.

P250

Studying Association of Null Genotype of *GSTT1* and *GSTM1* in Vitiligo Patients of Bhopal

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Vitiligo is an acquired, progressive, idiopathic and worldwide common depigmentation disorder prevalent among all age groups and both sexes with social stigma associated in India. Though melanocyte loss is responsible for Vitiligo, however the reason for this loss is yet to be found. It is emerging that genetic susceptibility and gene-environment interactions may be an important determinant for melanocyte loss. In other ethnic populations it has been shown that ineffective scavenging of oxidative stress in melanocytes due to null genotype of *GSTM1* and *GSTT1* may lead to melanocyte death. Hence we designed this hospital based pilot case-control study to evaluate association of null genotype of *GSTM1* and GSTT1 with predisposition to Vitiligo among patients from Bhopal. To evaluate association of GSTM1 and GSTT1 null genotypes with Vitiligo among patients from Bhopal and correlate the genotype with demographic parameters, disease type and progression. Genomic DNA extraction from peripheral blood (2ml) samples was conducted after obtaining informed consent and demographic and clinical information from 17 vitiligo patients visiting Dermatology OPD of AIIMS Bhopal and matched 26 Vitiligo free controls from the OPD and nearby areas of the hospital. Genomic DNA was amplified by PCR for GSTM1, GSTT1 and IFN (housekeeping gene) and the PCR products (273 bp, 459 bp and 173 bp respectively) were analysed on 2% agarose gels stained with ethidium bromide. Chi-square test was performed to analyse association of the genotype with the occurrence of Vitiligo In our study, gender, diet, tobacco habit and koebnerization did not significantlyassociate with occurrence of Vitiligo. Acrofacial followed by segmental type of Vitiligo was prevalent in cases. There was trend of family history of vitiligo and other autoimmune diseases though the association was not significant. Trend for null genotype for *GSTM1* was observed in patients compared to controls. Despite limitation of small sample size, trends observed in family history and *GSTM1* null genotype are notable. These findings will be validated as a part of DST funded study of analysing 15 polymorphisms in 10 genes regulating DNA repair and oxidative stress in 1000 subjects. This may assist in finding genetic marker associated with progression of disease.

P251

Effect of Anticoagulant: Can Heparin Produce Negative Bias in the Determination of Total Blood Calcium?

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Calcium is a vital element in the body which plays an important physiological role as it act as second messenger in many biochemical pathways. Accurate results of total and ionic calcium level plays a pivotal role in patient care and management. Measurement of serum calcium helps to identify many clinical disorders. Preanalytical condition including use of heparin during collection affect calcium estimation. The aim was to determine the effect of heparin on assay of total blood calcium for analysis of preanalytical error in collection of blood samples. This study was approved by Swami Vivakanand Medical Mission ethical Committee. Informed consent was obtained from the donors. Blood samples were obtained from the 10 subjects between 18-50 years age group including both genders. Data was analyzed using Deming regresion analysis, Bland and Altman method, paired t-test and Spearman correlation coefficients (r values). No significant changes were observed when plasma levels were compared with the serum values with P value of 0.56 and spearman correlation coefficients (r value) of 0.98. Deming regression analysis yielded the equation: 1.02 × (serum total calcium value) + 0.28 mg/dl. The bias value was -0.109 mg/dl (95% CI: -0.248 - 0.030 mg/dl) for total calcium levels assayed using either tube was acceptable. Study demonstrated that heparinized tubes can be used for determination of total calcium with an advantage fast processing of blood samples in an emergency laboratory setting. Moreover, single sample can be used for multiple purpose including both haematological and biochemical analysis.