Guest Lectures

Diagnostic, Therapeutic, and Prognostic Biomarkers in Infectious Diseases

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ABSTRACT

Biomarkers are important for diagnosis, monitoring treatment, and prognosis of infectious diseases. They help in diagnosis of bacterial *vs* viral infections; local from systemic infections; rule out sepsis; predict severity, outcome, and multiorgan failure; and monitor response to antimicrobial therapy. There are more than 100 biomarkers known, but only a few have sensitivity and specificity more than 90%. There are various types of biomarkers: Acute phase reactants, cell markers, receptors, cytokines, coagulation factors, etc. Immune markers: Monocyte chemoattractant protein-1 and tumor necrosis factor-alpha are associated with increased risk of death.

This presentation will focus on biomarkers in sepsis, pneumonias, prosthesis-associated infections, and management of antibiotic therapy.

Genetic Counseling in Inborn Errors of Metabolism

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ABSTRACT

Inborn errors of metabolisms (IEMs) are inherited disorders occurring due to a metabolic block because of deficiency of an enzyme or cofactor leading to metabolic derangements in the body. With better control of infectious diseases and malnutrition, as well as access to advanced technology for diagnosis, the knowledge of diagnosis and management of metabolic disorders is becoming more and more important. Although therapy is available for many of the IEMs, genetic counseling of family and prenatal diagnosis remains the mainstay of management of such families. Inborn errors of metabolisms can present at any age, from newborns to adult life. Phenotype can vary from acute illness with nonspecific signs and symptoms to chronic encephalopathy. The common presentations include neurological dysfunction presenting as acute encephalopathy, seizures, acute ataxia, and stroke like illness, acid base imbalance, hypoglycemia, cardiomyopathy and cardiac rhythm abnormalities, and acute liver disease. The chronic phenotype with a progressive course is seen in the storage disorders. These children usually present with psychomotor retardation with or without seizures, and evidence of other neurologic dysfunction like abnormalities of tone, extrapyramidal signs, etc. Full diagnostic workup followed by molecular analysis is essential for genetic counseling, diagnosis, and prenatal diagnosis of IEMs.

The presentation will focus on case-based approach to diagnosis and genetic counseling for common IEMs.

Essential Characteristics of a Good Laboratory Information System

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ABSTRACT

Use of laboratory information system (LIS) for management of laboratory information has become almost indispensable for current medical laboratories. Due to lack of professionals trained both in medical laboratory as well as information technology, the LIS used/created do not meet laboratory expectations. Moreover, there are efforts at national and international levels to provide standards for patient care. Such standards put requirements to be fulfilled for LIS used by hospital and laboratory. Development of LIS must take care of such requirements.

While a large hospital/laboratory can develop a LIS for their need, smaller laboratory needs to purchase them from outside. In any case, LIS needs to satisfy certain essential characteristics for being useful and to conform to the regional, national, and international standards. Without such essential characteristics, the LIS of the laboratory may become useless; and times obstruction to smooth functioning of the laboratory. First part of the lecture on essential characteristics of a good LIS system aims at discussing LIS-related requirements by ISO 15189, National Accreditation Board of Hospitals and Health care (NABH) standards for hospitals, and National Quality Assurance Standards for Hospitals (NQAS). The second part aims at discussing important desirable operational characteristics like correctness, integrity with Health Management Information Systems (HMIS), efficiency, security; some revision characteristics like flexibility, extensibility, modularity; and some transferability characteristics like interoperability and portability of a LIS system.

Intracellular Calcium: Its Universal Role as Regulator

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ABSTRACT

Calcium was discovered and named by Humphry Davy in 1808, being the metal element in limestone, chalk, and lime. But it was not until the end of the nineteenth century that calcium's unique role as a trigger of a wide range of physiological and pathological events began to emerge. All life requires calcium. It is required for the hard components in bone, teeth, and shells, has an important electrical role in excitable cells, such as nerves and the heart, and is required for blood to clot. But the role of calcium here is its universal role inside cells, triggering a wide range of events in all animals and plants, and many microbes. These events include nerves firing, muscles contracting, a heart beating, endocrine and exocrine secretion, egg fertilization, plant breathing, as well as cell defense, and even cell death. Disturbances in intracellular calcium play a vital role in many diseases, and many medications work either directly or indirectly on the intracellular Ca^{2+} toolkit. The key experiment is to measure the concentration of free Ca^{2+} in the cytosol, or an intracellular compartment, and correlate the change in free Ca^{2+} there with the cell event. Two methods are now used to achieve this – genetically engineered Ca^{2+} activated photoproteins, originally from a jelly fish or hydroid, and small organic or genetically engineered fluors. These have shown that the key feature of every cell is the Ca^{2+} pressure across the outer membrane of the cell. This pressure is generated because the cytosolic free Ca^{2+} in all animal, plant and microbial cells is submicromolar, even in the presence of 1 to 10 mm outside. A primary electrical, chemical, or biological stimulus generates a Ca^{2+} signal inside the cell. When this reaches its target, a high affinity Ca^{2+} binding protein, such as troponin C or calmodulin, causes the cell to cross the Rubicon, and an event occurs. Thus all the organs in our body – the brain, heart, leg and arm muscles, pancreas, liver, kidney, gonads, eyes, and the immune system – depend on this Ca^{2+} pressure. It is abnormalities in the Ca^{2+} signaling system that underlie much of the cell damage detected by tests in the clinical biochemist's laboratory. An important feature of the Ca^{2+} signaling system is its molecular biodiversity, giving each cell, each organ, and each individual organism, uniqueness, upon which, ultimately, natural selection depends. It is clear that the ability to maintain a very low cytosolic free Ca^{2+} was a very early process in the evolution of life, as high Ca^{2+} inside cells wrecks DNA and RNA, activates proteolysis, and causes protein coagulation. By the Cambrian explosion some 600 million years ago, most of the components of Ca^{2+} signaling toolkit were in place. The universal importance of Ca^{2+} in all cells depends specifically on the unique chemistry of the calcium ion. Yet there still much to learn, particularly on the role of Ca^{2+} in bacteria and archaea. The story of intracellular Ca^{2+} over the past 100 years is a wonderful example of how discovery, invention, and scholarship work together in science. The studies of thousands of creative scientists into Ca^{2+} have resulted in amazing revelations on how life operates, what goes wrong in disease, and have even created several billion dollar markets. As the Nobel Laureate Otto Loewi once remarked over 50 years ago: "Ja Kalcium, das istalles" – "Yes calcium, that's everything."

Advanced Glycation End Products and Its Role in Diabetic Complications

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BACKGROUND AND AIM

Plasma and tissue level of advanced glycation end products (AGE) has been shown to be associated with severity of diabetes mellitus and its complications. Interaction of AGE with its receptor (RAGE) induces signal transduction that culminates in vascular complications in type II diabetes mellitus (T2DM), the major cause of morbidity and mortality in diabetic subjects. Some functional polymorphisms of RAGE gene affects AGE-RAGE binding and signal transduction and is thought to be associated with the development of vascular complications in diabetic patients. In the present report, association and expression of RAGE gene and their polymorphisms have been investigated in relation to complications in T2DM patients. Also, AGE-mediated reactive oxygen species (ROS) generation and toxicity has been evaluated *in vitro* using peripheral blood mononuclear cells (PBMC) and human umbilical vein endothelial cells (HUVEC).

MATERIALS AND METHODS

A total of 820 subjects that include T2DM subjects without any vascular complications (n = 200), T2DM subjects with microvascular complications (n = 220), T2DM subjects with macrovascular complications (n = 200), and 200 healthy controls were enrolled for the study. Serum AGEs were estimated by enzyme-linked immunosorbent assay and spectrofluorometric assay. Three functional polymorphisms of RAGE gene, namely –374 T/A, –429 T/C, and Gly82Ser polymorphisms were determined in study subjects. DNA isolated from the enrolled subjects were genotyped by polymerase chain reaction-restriction fragment

length polymorphism (PCR-RFLP). Receptor AGE expression was determined by quantitative real-time PCR (qRT-PCR). The AGE-albumin was prepared by interaction of human serum albumin with D-glucose and their formation was characterized by lysine residue modifications and fluorescence properties. The AGEs-treated PBMC/HUVEC cells were tested for cell cytotoxicity (by MTT assay), DNA damage (8-OH-dG levels and Comet assay), apoptosis (annexin V-binding assay), and RAGE expression.

RESULTS

Serum level of AGEs was significantly higher in diabetic patients having vascular complications as compared with T2DM without complications ($p < 0.01$). Serum AGE level ≥1.52 µg/mL may be considered as risk factor for the development of vascular complications in T2DM patients. Mutant variant of –429T/C and Gly82Ser RAGE polymorphism was about three times more prone to develop macrovascular and microvascular complications respectively in T2DM subjects, while –374A allele showed reduced risk toward the development of macrovascular complications (odds ratio = 0.57, $p = 0.006$). Further, haplotype analysis also revealed that CTG haplotype was significantly associated with the development of macrovascular complications, while haplotype TAG was found to be significantly protective toward macrovascular complications in T2DM subjects. AGE-induced ROS generation was found to be mediated by AGE/RAGE interaction and involvement of nicotinamide adenine dinucleotide phosphate oxidase. We found 3.4-fold and 2.5-fold increase in olive tail movement in comet assay and 8-OH-dG levels respectively in advanced glycation end products-treated PBMC/HUVEC cells. The AGEs also found to induce apoptosis and RAGE expression. Co-treatment of antioxidants was found to ameliorate AGE-mediated damage.

CONCLUSION

The AGEs accumulation and presence of mutant genotypes of RAGE gene and its enhanced expression may be considered as risk factor for vascular complications in T2DM patients. The AGE plays a key role in the induction of oxidative stress facilitated through AGE/RAGE interaction and is responsible for genotoxicity and apoptosis.

Why lecture badly?

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ABSTRACT

In medical schools all over India, traditional teaching methodology is practiced wherein lecture forms a core teaching strategy. An average medical student attends more lecture than he sees patients. However, with the advent of e-revolution and availability of newer teaching methods like project-based learning, cooperative participating exercises (CPE), etc., the didactic lectures seems to be losing its place. Nevertheless, because of various reasons, including staff shortage and lack of viable alternatives, lectures are unavoidable and are going to stay well into this century. This talk deals with various vices and virtues associated with lecturing. The emphasis will be on incorporating good points of CPE into lecturing with the role of humor orientation scale, role play, clinical orientation, etc. The aim is to refresh approach to learning and making lecturing more refreshing for the students.

Job Satisfaction Status and Its Implications among Medical Biochemistry Teachers

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ABSTRACT

Job satisfaction is a positive personal perception toward work. It is a feeling of an individual toward his profession, may be positive or negative. Three theories have been proposed for job satisfaction: Content (Maslow's hierarchy of needs), Process (individual's perception), and Situational (characteristics and occurrences) theory. To measure job satisfaction level, two methods are used widely: Single global rating and summation of job facets.

Job satisfaction of medical teacher is a major factor having impact on quality of teaching and performance of the students. It is a complex phenomenon affected by multiple intrinsic and extrinsic variables. Intrinsic factors are recognition, achievements, opportunities of promotion, and personal growth. Working environment, salary, position, job security, and interpersonal relations are extrinsic factors. Job satisfaction is an outcome of appraisal of work and experience that depends upon individual's perception, departmental working conditions, and institutional dimensions. To ensure high-quality teaching in medical education system, job satisfaction of medical educators is the most important but least studied issue.

Medical biochemistry teachers perform dual role as teacher and laboratory physician in medical colleges. Also they are involved in administrative work and research activities. Since last two decades influx of medical graduates into field of biochemistry is increasing all over India. But their level of job satisfaction has never been studied. We evaluated job satisfaction/dissatisfaction status of medical biochemistry teachers from government and private institutes from different parts of India. Also the study is aimed at identifying factors influencing level of job satisfaction/dissatisfaction.

In this cross-sectional descriptive analysis, we surveyed job satisfaction under three facets: Work satisfactions, satisfaction with facilities provided, and professional growth by studying personal and job characteristics. Pretested questionnaire with Likert scale was the tool to collect data. During National Conference of Association of Medical Biochemists of India at Tirupati, 80 volunteers responded. Job satisfaction was compared according to designation, type of institute, and salary.

Among 80 participants, 64% were assistant professors, 17% associate professors, and 19% professors. Male respondents were 54% and females 46%. A total of 43% respondents were from government and 47% from private medical colleges.

Moreover, 62% people preferred to advise medical graduates to opt for MD Biochemistry. This is an important parameter reflecting job satisfaction. Only 27% participants had first choice of MD Biochemistry while joining the course and 28% would still like to change the profession.

We found positive correlation of higher level of posts with work satisfaction, facilities, and professional growth ($r = 0.442$, 0.281, and 0.52 respectively). Monthly salary also has positive correlation with these variables (0.508, 0.42, and 0.589 respectively). Type of organization, may be private or government, did not affect significantly work satisfaction, salary satisfaction, and professional growth among teachers. There is positive correlation of higher level of post with amount of salary, while this correlation was insignificant with satisfaction in laboratory services.

Job satisfaction is a multidimensional construct determined by number of variables. Job dissatisfaction is a direct threat to personal health and quality of life, leading to absenteeism from work, conflicts, students' performance, and indirectly quality of service to patients. Risk factors for job dissatisfaction are job insecurity, inadequate payment, poor working conditions, and promotional opportunities.

Employers should identify the predictors of job dissatisfaction of teachers and implement the strategies to increase it by recognizing their work, improving work environment, salary increment, and changing promotional policies. Medical teachers should be involved in the design and decision-making process of educational programs and their implementation so that they can become role models for learners. Although data from the present study is limited, hopefully it will serve as a basis for further studies to ensure to high-quality training to medical students by improving job satisfaction of medical teachers in India.

Quality Assurance in Clinical Laboratories

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ABSTRACT

In health care today, medical laboratories are key partners in ensuring and maintaining patient safety, and it has been seen that laboratory results influence approx. A total of 70% of medical diagnosis. Maintaining quality standards in the laboratory service plays a major role in ensuring the accuracy of these results, providing better patient care as a whole, and promoting excellence. While the absence of the same may lead to unreliable results, causing a delay in treatment, misdiagnosis, and an increase in cost due to a need for retesting.

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

International standard ISO15189, based on ISO17025 and ISO9001 standards, provides the basic requirements for establishing competence and serves as the bible for quality in medical laboratories. And while this serves as an excellent guiding principle, no matter how good the quality mechanisms are on paper, truly good quality cannot be achieved if theory is not translated into practice day-in and day-out.

There is a cost associated with quality, but are we cognizant of the fact that poor quality costs us even more? Quality costs can be offset by quality payoffs like enhanced reputation, loyal clientele, reduced system failures and machine downtime, less need for retesting for complaints, etc. However, there is no offset for medical implications that may be caused by poor quality, and its impact on not just the laboratories in question but on health care as a whole.

Implementing an efficient quality management system does not guarantee a 100% error-free laboratory, but it goes a long way in detecting errors that may occur commonly, and prevents them from recurring. It essentially puts us on the path to continuous improvement, and brings us closer to our vision of bettering health care facilities every day.

Thus, implementing and maintaining good quality standards in laboratories is no more a choice, as it is not just the ethical and moral duty of all laboratories to provide accurate, reliable results, but it is essential to all aspects of health care and the medical profession.

Sodium and Essential Hypertension

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ABSTRACT

Sodium is an important component of a healthy diet, but too much can cause high blood pressure, which is the second leading cause of kidney disease. A low-sodium kidney diet will be recommended by your doctor and a renal dietitian can help you make food choices that are beneficial to your health.

Primary hypertension (HTN) is fetal in origin ("*in utero*"). Renal developmental abnormality makes the individual susceptible for developing essential HTN. Low birth weight (intrauterine growth restriction) predicts adult onset HTN. Genetic factors contributing to essential HTN is minimal or nil.

Renal retention of salt and water is the prerequisite for the development of both primary and secondary HTN. The risk of developing salt and water retention is less in people with normal birth weight.

Salt-free diet/added salt restricted to 2 gm per day prevents both forms of HTN irrespective of birth weight (low or normal), age, ethnicity, presence, or absence of comorbid conditions, making "Hypertension Free World" – a reality.

An Insight into Laboratory Viewpoints on Thyroid Function Tests

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ABSTRACT

Thyroid function tests are of two types:

1. Tests that establish if there is thyroid dysfunction [thyroid- stimulating hormone (TSH), free T4 (FT4), free T3 (FT3)]
2. Tests to identify the cause of thyroid dysfunction, e.g., thyroid auto-antibodies.

1. Tests which establish if there is thyroid dysfunction (TSH, FT4, FT3):
   a. Thyroid stimulating hormone assay
      - The measurement of TSH in a basal blood sample by a sensitive immunometric assay provides the single-most sensitive, specific, and reliable test of thyroid status in both overt and subclinical primary thyroid disorders.
      - In primary hypothyroidism, TSH is increased whilst in secondary hyperthyroidism TSH is below 0.1 mU/L. However, abnormal TSH concentrations may be found in some euthyroid patients. Thyroid stimulating hormone alone is not a reliable test for detecting secondary and tertiary hypothyroidism.
      - It is essential that laboratories use a TSH method with a functional sensitivity of <0.02 mU/L. Prior to the introduction of a TSH method, the laboratory should validate the functional sensitivity quoted by the manufacturer. Quality assurance procedures should be in place to ensure that the functional sensitivity of the assay is regularly monitored.
   b. Free hormone assay
      - Laboratories should be aware of how their assay performs in various clinical situations, including thyroid disorders, pregnancy, nonthyroidal illness, certain medications (e.g., heparin, phenytoin, frusemide, carbamazepine, salicylate), and familial binding protein abnormalities, such as familial dysalbuminemic hyperthyroxinemia and thyroxine binding globulin excess or deficiency.
      - Free hormones can increase in some samples on storage, hence it may be necessary to freeze samples that cannot be assayed within 48 hours of collection.
      - Interference from antithyroid hormone antibodies is method dependent and laboratories should know how the presence of such antibodies would affect their assay

Many laboratories measure basal TSH as the initial test of thyroid function. This strategy might not be applicable when the pituitary axis is not intact or unstable. Also the use of first line TSH testing might go wrong in common situations like optimizing thyroxine therapy in the early months of treatment of newly diagnosed patients with hypothyroidism, diagnosing and monitoring thyroid disorders in pregnancy, and monitoring patients with hyperthyroidism in the early months after treatment. If clinical details are not available that allow the identification of the above categories of patient, then it may be prudent for laboratories to measure serum TSH and FT4 on all specimens rather than embark on a first-line serum TSH testing strategy.

REFERENCE RANGE

The method-related bias arising because of differences in standardization, the matrix of the calibrators, and specificity of antibodies should be compensated by the use of method-related reference ranges, where possible manufacturers reference ranges should be confirmed locally using an adequate population size of at least 120 ambulatory subjects.

- For TSH, reference ranges should be established using specimens collected between 0800 and 1800h (as TSH exhibits diurnal variation) and using 95% confidence limits from log transformed data. Alternatively, nonparametric methods of statistical analysis could be used to derive the reference range. The reference population should have no personal or family history of thyroid dysfunction, be on no medication known to alter TSH and have no thyroid antibodies detectable by a sensitive assay.
- Since TSH, free, and total thyroid hormones change during pregnancy, trimester-related reference ranges should be available.

"Nonthyroidal illnesses" or "sick-euthyroid syndrome":

Patients suffering from any of a wide range of chronic or acute nonthyroidal illnesses may show abnormalities in thyroid function tests even though they are clinically euthyroid. This has been described as the "sick-euthyroid syndrome." In the majority of these sick patients, TSH will be normal and thus provide the best guide of thyroid status. However, in some patients, TSH concentrations may be suppressed in the acute phase. This is explained by the fact that stress suppresses TRH. Total T3 and FT3 concentrations usually fall as a result of impaired tissue uptake of T4 and impaired conversion of T4 to T3.

- In hospitalized patients, a TSH <0.10 mU/L is at least twice as likely to be due to nonthyroidal illness as hyperthyroidism.
- In hospitalized patients an increased TSH is as likely to be associated with recovery from illness.
- Additional investigations, such as FT3 may point strongly to nonthyroidal illness as a cause of the abnormal results. A repeat sample after 3 weeks may show that the abnormal results have been transient and attributable to an acute illness or a specific treatment regimen.

2. Laboratory tests used to determine the cause of thyroid dysfunction
   a. Thyroid Peroxidase Antibodies (TPOAb)
      - These are present in the serum of patients with a wide range of immunologically mediated thyroid disorders (e.g., Hashimoto's thyroiditis, Graves' disease). They may also be found in a small proportion of apparently healthy individuals but the appearance of TPOAb usually precedes the development of thyroid disorders.

   b. Thyroglobulin Antibodies (TgAb)
      - These are present in the serum of patients with a wide range of immunologically mediated TgAb.
      - Antibodies to thyroglobulin (TgAb) are found in many patients with autoimmune thyroid disorders; however, in most circumstances TgAb measurements have no additional value over the measurement of TPOAb and need not be done if TPOAb is present. TgAb measurements may be helpful in patients with differentiated thyroid cancer. Because of marked differences in assay sensitivity, it should be noted that the absence of TgAb in one assay does not absolutely exclude their presence.
      - In iodine sufficient areas it is of no value to measure both TgAb and TPOAb in nonneoplastic conditions like thyroid disorders (e.g., Hashimoto's thyroiditis, Graves' disease). They may also be found in a small proportion of apparently healthy individuals but the appearance of TPOAb usually precedes the development of thyroid disorders.

The only reasons to measure Tg antibodies are: (i) In differentiated thyroid cancer to determine possible interference from these antibodies in immunoassays for thyroglobulin; (ii) serial measurements may prove to be useful as a prognostic indicator.

   c. Thyroid stimulating hormone Receptor Antibodies (TSH-RAb)

      The measurement of TSH-RAb is particularly useful in pregnancy and may also be helpful in the following situations:
      - To investigate hyperthyroidism of uncertain etiology
      - To investigate patients with suspected "euthyroid Graves" opthalmopathy
      - For pregnant women with a past or present history of Grave's disease
      - To identify neonates with transient hypothyroidism due to TSH blocking antibodies.

Other analytes like thyroglobulin, calcitonin can be also be included in the panel.

Thyroid dysfunction is an insidious condition with a significant morbidity and the subtle, and nonspecific symptoms and signs may be mistakenly attributed to other illnesses, particularly in postpartum women and the elderly. This speaks of the significance of sensitive and specific biochemical markers like TSH and free hormones assays' role in diagnosing the condition and antibodies' role in identifying the cause of the disorder.

The Pesticides: Boon or Bane

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ABSTRACT

Pesticides are an undeniable part of modern life, used to protect everything from flower gardens to agricultural crops from specific pests. In recent years, few environmental issues have aroused public concern as much as use of and exposure to pesticides, especially with respect to children's health. Every year, pesticides are estimated to cause tens of millions of cases of accidental poisoning, largely in the developing world. Many pesticides that have been banned or whose use has been severely restricted in industrialized countries are still marketed and used in developing countries. Developing world farmers are at special risk of harmful exposures due to the nature of the pesticides they use and the widespread lack of awareness of the risks they pose. Adequate safety measures are often not employed and pesticide poisoning is a frequent consequence. Under present conditions in many developing world nations, safe use of pesticides cannot be guaranteed. Current regulatory frameworks do not adequately account for the patterns of pesticide use in developing countries (e.g., mixing of pesticides, lack of protective clothing, high exposure levels). Some researchers have shown that synergistic effects occur when certain substances are mixed,

which causes them to become more toxic than on their own. When chemicals are mixed in an uncontrolled manner, chemicals that act similarly (e.g., cholinesterase-inhibiting pesticides) can have additive toxicity (cocktail effect), though individually they are considered below dangerous levels. So, at the time of mixing it is better to identify pesticides which do not have synergistic effects. An experimental study has demonstrated a cocktail effect for a mixture of five pesticides found in human food and their metabolism speedup the formation of genotoxic effects, such as DNA damage, synergistic neurotoxicity by a direct mechanism at the cellular level, and exposure to endocrine disruptors is especially harmful to fetus and young children, as the body's systems are particularly sensitive to hormones when they are developing and cause immunosuppression. Furthermore, the worse part is that farmers in developing countries routinely mix pesticides by hand without gloves and mostly they are barefoot, they do not wear protective clothes and masks, small children are directly involved in agriculture, including mixing and application of pesticides. Whilst such pest control plays an important role in modern medicine and agriculture, pesticide use is not without risk, so urgent action is required in order to reduce the health threats posed by pesticides and its use is done for good harvest without effecting the lives of people.

Biosynthesis and Extraction of Secondary Plant Metabolites

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ABSTRACT

Secondary plant metabolites represent the vast chemical diversity available in nature. Natural product research continues to be an important contributor to drug discovery. The starting materials for which are crude solvent extracts from source drugs.

The relationship between primary metabolism (biochemistry) and secondary metabolism (natural product chemistry) forms the basis of production of millions of secondary metabolites which are pharmacologically active constituents. The extracts may be used as a medicinal agent or may be further processed to be incorporated in any dosage form or fractionated to isolate individual chemical entities as modern drugs. The purpose of standardized extraction procedures for crude drugs is to attain the therapeutically desired portion and to eliminate the inert material by selective solvents. Extraction procedure contributes significantly to the final quality of herbal drugs.

The lecture will present an understanding of the building blocks and specific metabolic pathways involved in the production of a wide array of secondary metabolites and also the basis of extracting secondary metabolites and the technologies available for their extraction. The various processes of production of medicinal plant extracts will be discussed with reference to hepatoprotective plant constituents.

Cardiac Biomarkers: Feasibility, Utility and Objectivity!

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ABSTRACT

Presently, cardiovascular disorders are toward the increasing trends. Role of cardiac biomarkers (CB) becomes pivotal in diagnosis, risk stratification, and treatment of patients with cardiac injury. Cardiac biomarkers are biomolecules that are released into circulation in case of myocardial/cardiac injury like myocardial infarction, unstable angina, heart failure, or myocarditis.

Initially, SGOT and lactate dehydrogenase were used in diagnosis of myocardial infarction. But, later in 1972, use of creatine kinase-MB revolutionized the process of diagnosis of cardiac patients, which was then taken over by Troponin (cTn) assays in 1989. Endeavors of better understanding of cardiac disease processes paved the way for introduction of newer CB and expanding the spectrum of diagnosis of cardiac injury other than myocardial infarction like pulmonary embolism, acute heart failure, myocarditis etc. Newer CBs can be categorized as: (i) Biomarkers of inflammation like C-reactive proteins, myeloperoxidase, soluble CD 40, pregnancy associated plasma protein A, interleukin (IL)-6. (ii) Biomarkers of ischemia like ischemia-modified albumin, glycogen phosphorylase enzyme BB, free fatty acids. (iii) Biomarkers of hemodynamic stress including brain natriuretic peptides. Progress in the feasibility and specificity of measuring troponins is driving a trend toward earlier point of care (POC) technology implementation in decision-making and risk stratification. However, the panoply of putative CB is also contributing to the emergence of cardiac POC. The cumulative information derived from a POC multimarker panel can be superior to traditional cTnI laboratory testing. The ultimate goal of cardiac diagnosis is prevention of even a minor infarction, and, therefore, only biomarkers preceding necrosis can satisfy clinical needs. Furthermore, certain aforementioned biomarkers have shown promises for early diagnosis and prevention and certain have been approved by Food and Drug Administration (FDA). Moreover, full potential of these emerging biomarkers can only be realized when they will be judiciously multiplexed into POC platforms. However, simultaneous advances in assay technologies are also required so that low levels of these markers can be exquisitely detected and earliest medical interventions are employed.

Are We addressing the Learning Style of Students?

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INTRODUCTION

The transition from school to university undergraduate education is challenging for students due to a quantum leap in the volume of content. A medical student's program of study and course content is much more than other undergraduate courses. The vastness of medical course-work to be covered within a limited timeframe can be very frustrating. The discomfort of assimilating this vast course content is worsened due to the disparity between learning and the delivery of instruction. The compatibility between teaching style of instructor and learning style preferred by the students helps the latter to understand the concept, retain information for a longer period, and apply it more efficiently. Different persons perceive and process the information differently, and accordingly make decisions and define what is important. Learning style is the individual's best way to accumulate, understand, and apply gathered knowledge in a specific manner.

TOOL FOR ASSESSMENT

Fleming and Mills defined four modes of learning and these are: Visual (V), Auditory (A), Read-Write (R), and Kinesthetic (K) (hence the acronym VARK). The visual learners are those who typically learn by depiction of information in maps, spider diagrams, charts, graphs, flowcharts, labeled diagrams, and all the symbolic arrows, circles, hierarchies. The auditory learners conceptualize information from lectures, group discussion, radio, email, using mobile phones, speaking, web-chat, and talking things through. The read-write learners prefer printed text material, i.e., PowerPoint, textbooks, the Internet, Google and Wikipedia, lists, quotations and words, words, words… while the kinesthetic learners understand best through moving, acting, performing, practicing the activities, and using smell and touch (tactile sensory ability). So, demonstrations, simulations, videos and movies of "real" things, as well as case studies, practice and applications help them to assimilate information.

Students can use all these modes of learning but one mode can be dominant in some and these are termed as unimodal learners, e.g., visual/auditory/read-write/kinesthetic learners. Some others might prefer using a combination of two, three or all four modalities and are accordingly termed as bimodal, trimodal, and quadmodal respectively.

FEEDBACK FROM STUDENTS

We conducted a study on 1st year MBBS students using VARK questionnaire designed by Neil Fleming to assess their preferred learning style for three consecutive years. It was found that majority (80, 71, and 70%) preferred multimodal approach. Out of the four, each modality accounted for 22 to 28% of total responses, meaning thereby that all modalities play an almost equal role in learning by the students. This also implies that if we teach using only one modality, e.g., traditional lecture format (A), we are catering to only a small fraction of students. Incorporation of other modalities in teaching will address the needs of more and more students.

HOW DO WE ADDRESS THIS SITUATION?

Traditional lecture format (auditory) is used by educators because of relative ease permitted by this method to pass information, the need to cover large course content in a limited timeframe, their justification of following a traditional way of teaching as a time tested method, and their unwillingness to experiment with newer techniques.

The idea behind teaching the basic subjects in the beginning of MBBS is that the student should be able to understand the basic concepts and apply this knowledge to understand the other subjects, or any clinical situation. For this to happen in true sense of words, the subject has to be made more interesting, clinically relevant, and not as volatile as people would like to believe. To achieve this, there is a need to utilize this information about learning modalities of our students and modify the teaching of the subject accordingly. The time constraint of 10-month teaching and the vast course is a valid limitation. Yet it is felt that if all of us come together and try modifying our teaching at individual level to incorporate one or more modalities in different topics, we can share the experience at any forum and disseminate the same to our colleagues. This joint effort will go a long way in making the subject more assimilable.

Risk Management in Medical Laboratories

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ABSTRACT

Risk management in medical laboratories is a new subclause in ISO 15189:2012 document under clause number 4.14.6. It is documented as "The laboratory shall evaluate the impact of work processes and potential failures on examination results as they

affect patient safety, and shall modify processes to reduce or eliminate the identified risks and document decisions and actions taken." Being a new subclause, there is lot of apprehension and lack of clarity on this topic.

Risk is defined as "The effect of uncertainty on objectives, whether positive or negative." For health care, risk is generally understood to mean the chance of suffering or encountering harm or loss to the patient. It broadly consists of two parts: Measurable and immeasurable risk. Risks are present in all three areas viz, preanalytical, analytical, and postanalytical areas.

Risk management is described as the systematic application of management policies, procedures, and practices to the tasks of analyzing, evaluating, controlling, and monitoring risk. To achieve this, each laboratory should design its own risk management plan by which it should be able to analyze the process to identify the risk, assess the level of risk based on severity, and probability with grading, control the risk, and monitor and review controls.

Based on this, one can classify risk as low, medium, high, extreme and will have action plans like elimination, substitution, redesign, isolation, etc. Action taken should be evaluated by monitoring its effect. The lab should develop detailed responsibility matrix for risk management and have to implement this effectively with frequent monitoring.

Effective risk identification and its management helps reduce laboratory errors to almost nil, thus helping greatly in reliable laboratory reports and better patient management.

Plasma Dyscrasias: A Paradigm Shift in Diagnosis and Monitoring with Serum Light Chains Assay

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ABSTRACT

The advent of using serum light chains (SLCs) assay of kappa and lambda in diagnosis and monitoring of plasma cell dyscrasias (PD) has brought about a paradigm shift in evaluation and guidelines of treatment. A combination of conventional laboratory procedures, such as electrophoretic studies, urine assays, and bone marrow film examination, has now added into its panel the quantitative measurement of kappa and lambda in the serum, and thereby to a large extent subjects of monoclonal gammopathy of undetermined significance (MGUS) eliminated the 24-hour collection, where compliance is low.

Over a period of 5 years, the laboratory has received 800 samples annually for these assays and when studied retrospectively with clinical correlation a lot of gaps of understanding of clinical presentation of PD was eased and also more relevantly, challenges the interpretation along with the conventional studies.

The methods used were: (1) SLC – binding site, processed on Beckman Coulter and SPA Plus; (2) Electrophoretic pattern (SPE, IFE, UPE) on Helena/Alere; (3) Chemistries on Beckman Coulter Clinical systems – Synchron Cx and Unicel DxC 800; and (4) Hematological parameters on Sysmex XT 4000i; (5) Bone and tissue biopsies with respective stains.

- Thirty-five subjects of autologous bone marrow transplant on regular follow-up for complete remission or relapse have shown clinical benefits with the assay. Three subjects of MGUS on regular follow-up were recently diagnosed as active disease with only grossly altered ratio.
- Complicity of altered SLC ratio in diagnosed subjects on treatment with no clinical signs or symptoms of relapse and normal hematological and electrophoretic studies caused interpretative difficulties.
- Four cases with significant IFE patterns along with hematological parameters fulfilling criteria for PD showed no altered ratio.
- Eight cases of non-Hodgkin's lymphoma exhibited an altered ratio of SLC with correlating electrophoretic pattern.
- A distinct and important observation is that of renal impairment and interpretation of SLC. A single cut-off is not appropriate and hence cut-offs in correlation with creatinine values and estimated glomerular filtration rate (eGFR) need to be established. Renal impairment with elevated light chains has brought in an entity of renal gammopathy of undetermined significance. Correlation with eGFR is presently in process.
- Assay in critically ill or hospitalized patients showed grossly altered ratio with normal electrophoretic patterns, causing rethink on timing of sample.
- Three cases of retroviral infection had grossly altered ratio.

Serum light chain assay with altered ratio is indeed a significant parameter in the study of PD, and in our study, has been very relevant for monitoring the disease and equally important in diagnosis. But it has also shown variation in other scenarios making it, at times, an obstacle in interpretation. Periodic literature review in establishing one's own population and laboratory reference intervals is a necessity. It should always be remembered that SLC should be used along with laboratory and clinical correlation and never in isolation.

Vitamin D Immunity and Immunological Disorders

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ABSTRACT

Vitamin D, the prohormone on activation in the body, leads to its metabolic effects. Presently, vitamin D is so strongly associated with its effects on bone formation, including the calcium and phosphate metabolism, and that bone and vitamin D are like synonyms. Liver and kidneys play an essential role in this physiology.

However, studies in the last few decades have revealed the very important extraskeletal effects of vitamin D. The finding of extrarenal expression of enzyme CYP 27B (1-alpha hydroxylase) as well as of vitamin D receptor in many tissues and cell types establishes these extraskeletal effects. Presently, almost all tissues are known as the target tissues for vitamin D, e.g., heart, brain, skin, bowel, gonads, prostate, breast, parathyroid gland, etc.

Latest emerging view is that calcitriol is an immune-modulator. All types of immune cells monocytes, macrophage, DCs, T and B lymphocytes are influenced by vitamin D. Their basic activities are modified, leading to several effects; e.g., vitamin D is known to decrease the inflammatory response, enhances the antimicrobial effects, suppresses autoimmunity, etc. Alteration of these functions is seen in many immunological disorders.

Vitamin D has long been known to be beneficial in many diseases. During the days when there was no definitive treatment of tuberculosis, sunshine was an important part of overall regimen of recuperation in sanatoriums. The antimicrobial effect of macrophages in enhancing the expression of antimicrobial peptides like cathelicidin and beta-defensins is well known. The association of vitamin D with several immune disorders, particularly the autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, type I diabetes mellitus, inflammatory bowel disease, autoimmune thyroiditis, etc., is well known. The role of vitamin D in immunological disorders, including their pathogenesis and therapy, is highlighted in the lecture.

Novel Biomarkers of First Trimester Prediction of Pregnancy-induced Hypertension

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INTRODUCTION

Pregnancy-induced hypertension is a multiorgan and heterogeneous disorder and is one of the major causes of maternal and fetal mortality and morbidity worldwide. Current diagnostic criteria is applicable after 20th week of gestation, which includes hypertension (systolic blood pressure > 140 mm Hg and diastolic blood pressure > 90 mm Hg) and proteinuria (> 0.3 gm/24 hours). Pregnancy-induced hypertension aggravates very quickly from minor hypertension to life-threatening manifestations, such as preeclampsia (PE) and eclampsia (E). In severe cases pregnancy-induced hypertension can lead to other adverse obstetric outcome, such as intrauterine growth restriction (IUGR), intrauterine death (IUD), still birth, and premature deliveries.

The pathogenesis starts much earlier than the appearance of symptoms. If we could detect the underlying pathogenesis earlier in first trimester, we can predict the women at risk of developing pregnancy-induced hypertension and give them appropriate preventive care.

AIM

This study is aimed to identify a biomarker or combination of biomarkers for the prediction of pregnancy-induced hypertension in the first trimester

MATERIALS AND METHODS

In the present study, we prospectively examined 1287 women. All women were enrolled in the first trimester (11 + 0 to 13 + 6) during their first visit to the antenatal outpatient department of Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital, New Delhi and followed till delivery. Maternal factors like mean arterial pressure (MAP) and body mass index (BMI) were recorded. Serum levels of PAPP-A and Free –hCG were analyzed. Women who develop hypertension were treated as cases and women without hypertension who gave birth to a healthy neonate were taken as controls. In addition to PAPP-A and Free –hCG, we have analyzed serum samples of hypertensive women and their gestational age and sample storage time matched controls for tumor necrosis factor-alpha (TNF-α), interferon-gamma (INF-γ), and endothelin. Statistical analysis was done in Statistical Package for the Social Sciences using appropriate tests.

RESULTS AND CONCLUSION

All 1287 singleton pregnancies were followed till delivery. We excluded 274 women for the reason of lost to follow-up [n = 62 (5.06%)], other adverse outcome, such as gross congenital anomaly, still birth, IUD and IUGR [n = 112 (9.14%)]. In the remaining

1225 women, we found 69 (5.6%) cases of gestational hypertension, 57 (4.6%) cases of PE, and 3 (0.2%) cases of E. There were 984 women who remained normotensive till delivery and gave birth to clinically healthy neonates.

Among maternal factors, MAP ($p < 0.001$) and BMI ($p < 0.001$) were significantly high in cases as compared with controls. Among maternal serum markers, PAPP-A ($p < 0.001$) was low in cases as compared with controls, TNF-α ($p < 0.001$), INF-γ ($p = 0.005$), and endothelin ($p = 0.01$) were significantly high in cases as compared with controls. After multiple logistic regression BMI, TNF-α, and INF-γ were found to be associated with the prediction of pregnancy-induced hypertension. Our study is a large sample size prospective study that reveals the potential roles of these maternal factors and biomarkers in first trimester prediction of pregnancy-induced hypertension. This has the potential of being translated to patient care.

Association of Proinflammatory Milieu and Hypovitaminosis D with Insulin Resistance in Women with Polycystic Ovary Syndrome

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, presenting in up to 15% of this population, characterized by hyperandrogenemia, oligo or/and anovulation, and polycystic ovaries. Women with PCOS display greater degrees of insulin resistance (IR) and hence are at an increased risk of type II diabetes.

The mechanism of this IR is still not clear and chronic inflammation and hypovitaminosis D may contribute significantly to its pathogenesis. Proinflammatory cytokines and their expression regulatory elements as well as vitamin D receptor regulate genes that are crucial for glucose metabolism.

MATERIALS AND METHODS

Fifty women diagnosed with PCOS meeting the inclusion criteria (Rotterdam revised criteria 2003) along with 50 age- and body mass index-matched controls were recruited for the study.

Fasting venous blood samples was collected from study subjects on days 2 to 5 of menstrual cycle for hormonal assays and interleukin 6 and vitamin D levels. Vitamin D receptor gene polymorphism (FokI) gene and IL-6 gene –174G/C promoter polymorphism were studied by polymerase chain reaction-restricted fragment length polymorphism.

RESULTS

Serum IL-6 levels were significantly higher in the cases as compared with controls. GC genotype was significantly associated with IR as demonstrated by significantly elevated HOMA-IR values. Vitamin D was significantly lower in cases (16.6 ± 1.26) as compared with controls (21.9 ± 1.91) and showed positive correlation with HOMA-IR, testosterone levels, and free androgen index.

CONCLUSION

Our study demonstrates a plausible role of proinflammatory cytokines and hypovitaminosis D in the etiopathogenesis of IR as well as hyperandrogenemia in PCOS, thereby highlighting the potential therapeutic implications.

Ensuring Analytical Quality

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ABSTRACT

For ideal functioning in all phases (preanalytical, analytical, and postanalytical), a testing laboratory requires policies and procedures in place, which are commensurate with its infrastructure. The analytical phase comprises all the steps in the methodology of testing and quality control procedures. The process starts with choosing suitable equipment and the reagents, and culminates with the generation of a good quality result within a clinically acceptable and effective timeframe. Whether one's equipment is semi-automated or fully automated or whether one uses manual methods depends on the basic amenities in terms of time, space, finances, and needs of the clients, i.e., the doctors and the patients. In accordance to the analyzing platforms, the reagents and quality control (QC) material as well as QC procedures can be identified. Whether to use commercial IQC material or retained samples; whether to use the manufacturer's mean or the lab mean; what statistical analysis to perform; how to plot the Levy Jennings charts; how to assess and review QC data (IQC or EQA) – these are the questions that are the backbone of one's quality assurance policies and which are inherently important to achieving the ultimate goal – good quality of the reports. Hence, careful planning and implementation is required for the analytical phase.

Verification of New Analyte in Clinical Biochemistry

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AIM

There is a great demand for quality in health care services these days. So each medical laboratory has responsibility to ensure the best quality of their test results. There are different ways of achieving this end result, and compliance with ISO 15189 has been accepted as the optimal approach to assure quality in medical testing. First step to check analytical performance when some new analyte or analyzer is introduced into clinical laboratory is verification or validation process as per ISO 15189. So our aim here is to discuss the minimum requirement for verification of a new analyte in clinical biochemistry.

MATERIALS AND METHODS

Plasma glucose has been chosen as an example to demonstrate verification process. Precision, accuracy, and linearity dilution check study has been done as part of procedure. Coefficient of variation (CV) percentage has been calculated and compared with manufacturer provided CV%.

RESULTS

The achieved CV% comparable with manufactured CV% and also acceptable as per standard guidelines.

CONCLUSION

Verification of a new analyte is pursued as first step in lean total quality management. It also helps in ensuring patient safety by ensuring quality results.

Root Cause Analysis and Corrective and Preventive Action Management in a Clinical Laboratory

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ABSTRACT

An important aspect of total quality management is identification of nonconformances (NCs) followed by comprehensive occurrence management, which comprises largely of corrective action (CA) and preventive action (PA). These processes, commonly known by the acronym CAPA, ensure that accurate and high-quality results are achieved by the lab. Corrective action is an activity used to stop recurrence of NCs, while preventive action is a proactive process that prevents potential NC's from occurring. Both CA and PA are defined in clauses 4.10 and 4.11 respectively in ISO 15189:2012.

To ensure the effectiveness of CAPA, it is imperative to first investigate the root cause of failures/errors. This process is known as "Root Cause Analysis," which facilitates system evaluation as well as analysis of the need for CAPA. Root cause analysis (RCA) is even more useful for near-miss scenarios and helps in tracking and trending of data. Corrective action identifies the action needed to correct the cause of the identified errors followed by permanent elimination of these causes. Preventive action would then be aimed at determining potential problems before they occur to ensure that they do not happen. The effectiveness of CAPA can be evaluated at regular intervals by the systematic approach of plan-do-check-act cycle.

In conclusion, an efficient and well-planned CAPA helps laboratories to resolve the errors in their quality systems by evaluating both the nonconformance and in-conformance data. Therefore, CAPA system is a great management tool, which forms the basis of a continuous improvement plan essential for satisfying the regulatory requirements.