

**Syllabus for the Course of
Membership of Indian Board of Clinical Biochemistry
(MIBCB)
under the guidance of Association of Clinical Biochemists of
India (ACBI)**

Introduction

Clinical chemistry is the medical discipline devoted to obtain, explore and employ chemical knowledge and chemical methods of investigation, in order to procure knowledge about normal and abnormal chemical processes in man. These processes are studied on a general level, in order to get insight into human health and disease, and on a patient-specific level for diagnostic or monitoring purposes. One of the main tasks of the clinical chemist is direction and supervision of a laboratory department in a hospital or health service, where his role involves bridging the gap between rapidly developing laboratory technology and the growing knowledge on characteristics of disease. He must possess fundamental biochemical knowledge and have the ability to use this knowledge most appropriately as applied to clinical requirements, i.e. diagnosis of disease and planning and monitoring of therapy. Apart from providing a competent laboratory service, the clinical chemist must be able to function as a consultant to his clinical colleagues and liaise with them in the interpretation of laboratory results. His advice and professional consultation have at least three aspects, i.e. choosing the most appropriate laboratory investigation in a certain case, ensuring that the analyses are performed in the best possible way and correctly reported and, finally, providing information and (most important) interpretation on the significance and consequences of the laboratory data obtained.

Clinical chemistry

Clinical chemistry / Clinical biochemistry is a scientific discipline within medicine. It includes the analysis of body fluids, cells and tissues and interpretation of the results in relation to health and disease. The discipline encompasses fundamental and applied research into the biochemical and physiological processes of human and animal life, and application of the resulting knowledge and understanding to the diagnosis, treatment and prevention of disease. The scope of clinical chemistry is not solely confined

to laboratory activities as such, but in daily practice is strongly interrelated with patient care and treatment. Moreover, medical laboratory activities apart from being of purely chemical nature, imply to some extent the practice and study of biochemistry and molecular biology, haematology, immunology, microbiology. Detailed knowledge on the application of chemistry and molecular biology, both in diagnostic medicine, monitoring of therapy and in pathophysiology is indispensable.

Quality assurance

Safeguarding and protecting the public against misuse of medical laboratory investigations are important features of good laboratory practice and reliable laboratory diagnostics. Adequate laboratory management and scientifically based quality assurance procedures must be incorporated in the production and management of data in medical laboratories.

The clinical chemist

Professional interpretation of data obtained is an essential task of the clinical chemist for which he should be trained appropriately. The post-graduate study should provide an in depth knowledge of the chemistry of disease and the procedures and analytical techniques used in a medical laboratory. It is important that there should be a commitment to research and development. The object being to produce a person competent in laboratory procedures with a sound knowledge relating to the subject and able to interpret and impart laboratory findings and their implication, in consultation with colleagues.

Responsibilities of a Clinical Biochemist

The candidate to the Clinical Biochemistry training needs a background of knowledge in biochemistry, physiology, analytical chemistry, biophysics, molecular biology, immunology and genetics; they need a general knowledge of anatomy, histology, pharmacology (toxicology), pathology, hematology, microbiology, biostatistics and epidemiology. They must acquire knowledge of the common clinical disorders and understand the basic principles of laboratory utilization in the diagnosis and management of disease. They will require critical appraisal, management, budgetary, and administrative skills and knowledge of automation, electronic data

processing, and laboratory information systems. Ultimate responsibility for setting standards in the clinical chemistry laboratory service rests with the clinical biochemist. He should be able to offer advice on methods and instruments when requested by laboratories lacking a clinical biochemist; serving on inspection and accreditation teams; assisting with research protocols. In consultation with the responsible physician and in case where it is warranted, monitoring the clinical action taken on the basis of laboratory data provided to the physician and the consequences for the patient. He should participate in continuing education and maintenance of competence programs, keeping pace with changes in all areas of laboratory medicine, in order to understand, apply and develop the new technologies it may require.

The prime objective in the education of clinical biochemists is to impart an understanding of the appropriateness of a laboratory test for a particular patient at a particular time. They must be familiar with the meaning of sensitivity and specificity of tests and the importance of prevalence in determining the predictive value of a test result. Trainees should know the details of the various tests of organ function, as well as the laboratory determinations involved, the relative merits of multiphasic screening, organ profiling vs cost effective testing protocols. They should know the biochemical and pathological mechanisms of tissue injury, as well as the factors underlying the tests and the principles of management of the common clinical disorders. Clinical biochemists must be able to advise on the choice of tests when necessary and on the interpretation of laboratory results when appropriate. They are also responsible for setting the standard of patient care through the diagnostic and monitoring services of their laboratory and should assist the clinical staff in practicing effective laboratory utilization. They will monitor the action taken on the basis of information provided to the physician and the consequences for the patient. They should take the initiative in introducing new laboratory procedures and deleting those that have outlived their usefulness. They should know the factors that may affect biological variation, and those that may interfere with the analytical procedures, such as drugs and dietary constituents.

Teaching in clinical chemistry and laboratory medicine has undergone a dramatic change in recent years, mainly because of the introduction of evidence-based medicine, problem-based learning (PBL) and new technology and inclusion of clinical cases in the teaching material.

Trainees should understand the principles of analysis, be able to set up and operate the instruments, know their inherent errors, general maintenance, defects and potential problems, and be able to "troubleshoot" or assist repair personnel. They should conduct experiments under the direction of their supervisor to demonstrate the precision, accuracy, detection limits and linearity of response, range of application, running costs and cost-effectiveness of selected instruments. They should understand the criteria for instrument selection.

To summarise, the aim of the course is to impart training to graduates, to render them proficient to:

- a) perform the medical biochemical techniques
- b) interpret the results of various tests
- c) supervise the medical biochemistry laboratories
- d) Identify biochemical diagnostic agents or tests useful in diagnosis and monitoring response to therapy
- e) Interpret the clinical significance of biochemical lab test results

Name of the Degree

The name of the degree shall be Member of Indian Board of Clinical Biochemistry (MIBCB).

The Indian Board of Clinical Biochemistry (MICCB) is constituted under the provisions and directions of the Association of Clinical Biochemists of India (ACBI)

The degree will be given under the seal of the Association of Clinical Biochemists of India (ACBI).

In USA and UK, it is common that professional bodies are giving the degrees of higher education, such as MRCP, FRCPath etc. Similarly, the ACBI could take up a professional course in Clinical Biochemistry.

In course of time, ACBI should try to get the course affiliated with any national universities of India or abroad, or with an international professional body.

Need for the course

Although there are lots of Universities and Colleges in India, imparting MSc degrees in Biochemistry, a specialized course in Clinical Biochemistry is still lacking. There are few courses run by selected medical colleges, giving MD degree for MBBS graduates and MSc degree of Medical Biochemistry for Science graduates. However, seats for this course are very limited. A course on Clinical Biochemistry is not available anywhere in India.

Benefits

Those who pass the examination will be able to man and supervise the activities of the clinical laboratories. At the moment, the availability of seats to various MSc / MD courses in the universities is limited, and that too the speciality of clinical biochemistry is not emphasized. The number of positions available in the clinical laboratories in the country far exceeds the availability of suitably qualified persons.

Those who are undergoing regular MD / MSc courses can also take up this course, as it will help in their studies.

Eligibility to be admitted to the Diplomate course:

- a) Candidates with PhD (with thesis on biochemistry or clinical biochemistry), and having 3 years of experience in a clinical biochemistry lab, after obtaining the degree.
- b) Candidates with MD (biochemistry), and having 3 years of experience in a clinical biochemistry lab, after obtaining the degree.
- c) Candidates with MSc (in biochemistry having 2 year course or medical biochemistry having 3 year course), plus 5 years of experience in a clinical biochemistry lab, after obtaining the degree.
- d) Candidates with MBBS and having 5 years of experience in a clinical biochemistry lab, after obtaining the degree.

Students should be currently employed in clinical biochemistry laboratory at the time of enrollment in the Diplomate course, and should continue to work in a clinical biochemistry lab till the completion of the Diplomate course.

Teaching faculty

Faculty will be drawn from senior professors of Biochemistry preferably working in clinical biochemistry lab in medical colleges or in specialized laboratories. They should have good experience in clinical biochemistry lab. The College (ICCB) will appoint the teachers. Initially, professors who are coming forward will give the charge of developing different modules, and also conducting the modules at fixed time intervals. They will be given suitable remuneration, as fixed by the ICCB or by the EC of the ACBI.

General concept of the course:

The duration of the course shall be minimum 2 years; and maximum 4 years. The total course is divided into 6 modules, each of 4 months duration. Each module is comprised of 5 topics; thus the whole course will have 30 topics. At the end of each module, there will be an examination on the 5 topics of that module.

The course will be mainly through correspondence. Students will be sent the study material of each topic by email (or CD) every 21 days, and the students will send back the answers back. The answers are assessed.

There will be some virtual classes; one professor will take a class on a particular topic at the notified time, so that students can see it.

Contact Classes

Further, there will be 35 direct contact days (4 contact classes, each of 8-10 days duration, conducted every 6 months). The contact classes will be assigned at specific centres in India. Preferably, contact classes can be taken at 4 regions of India. These are mainly to demonstrate the working of various automated specialized laboratories, and to get hands on experience. At the end of the contact classes, there will be one examination by the professor organizing that particular contact classes.

Moreover, it is compulsory for the student to attend the preconference professional course, preconference workshops and regular conference sessions during the annual conferences of the ACBI; this will give further 5 days x 2 years = 10 days. Attendance is compulsory. These students will be preferentially selected for preconference workshops. Thus there will be a grand total of $35 + 10 = 45$ contact days during the 2 year course.

In short, there will be 30 theory (correspondence) topics, and 45 contact days (including ACBI conferences) during the 2 years.

Dissertation

It is absolutely necessary that the student should have a general knowledge on conducting research. He / she should be familiar with the research methodology, ethics in research, application of biostatistics in research, how to do literature survey, etc. Each student will be assigned a dissertation work in the field of Clinical biochemistry. Dissertation work can be carried out by the student either at his own laboratory, or at a national laboratory. The dissertation will be evaluated before the final examination.

Instead of dissertation, the student can take up his own research and publish. One publication in a standard peer-reviewed journal will be equivalent to the dissertation.

Candidates having PhD or MD qualifications are exempted from dissertation, because they are already familiar with the research methodology.

Carry over system

A student is supposed to pass all the 5 topics at the modular examination. **The candidates can move to the second and third semesters of the course, irrespective of whether they cleared the exams of the previous modules. However, before entering to the fourth module, all three previous module examinations should have been cleared.** In other words, only by completing all the first 15 topics he / she can enroll in the 4th module. Then from the 4th module onwards such carry over system is allowed till the completion of the whole course. The total course duration should not be more than 4 years.

Examination system:

Marks will be allotted to

- a) Marks obtained in the end-topic examinations. This is done through correspondence. Marks will be based on the answers as well as the

- punctuality of the student in sending back the answers, equivalent to attendance.
- b) Performance of the student through the contact hours
 - c) Attendance of the annual conferences of the ACBI, and
 - d) Examinations at the end of each module (module examinations).

Module examination will be on-line, conducted every 4 months, at specified dated and time at specified centres throughout India. Questions will be generally of MCQ type. There will be 6 module examinations, each carry 100 marks. For each module examination, there will be 100 MCQ, each correct question carrying 3 marks, and each negative answer carrying minus 1 mark. Duration of each module examination will be 100 minutes.

Marks are assigned as follows

End topic examination : 30 topics x 4 marks	= 120 marks
Contact, 35 days, 4 contacts (4 x 25 marks)	= 100
Attending 2 annual conferences =10 days(2 x 15 m)=	30
Module examinations (6 modules x 125 marks)	= 750
Total	= 1000

For a pass, the student should get a minimum of 80% in the total 1000 marks, plus 80% attendance (in contact classes and ACBI conferences).

Certificates will be given to the candidate only after successfully completing the dissertation work or getting one publication in a peer reviewed journal. There is no separate mark for the dissertation. But it will be examined by an examiner, and get approval that it has the minimum standard. (Candidates having the qualification of PhD and MD are exempted from dissertation work).

Summary of Syllabus (30 correspondence courses + contact courses)

Module 1

Topic 1.Biomolecules

Topic 2.Lab instruments

Topic 3.Enzymes**Topic 4.Diagnostic enzymology, including markers****Topic 5.Metabolism of amino acids and proteins****Module 2****Topic 6.Estimation of proteins, electrophoresis****Topic 7.Metabolism of carbohydrates, TCA cycle****Topic 8. Carbohydrates continued****Topic 9.Liver Function Tests****Topic 10. Kidney function tests****Module 3****Topic 11. Hb metabolism****Topic 12.Metabolism of lipids****Topic 13.Cholesterol, lipid profile****Topic 14.pH, acid base balance, Electrolyte balance****Topic 15. Pharmacology, Toxicology, Therapeutic Drug Monitoring****Module 4****Topic 16.Endocrinology****Topic 17.RIA, ELISA, hormone estimations****Topic 18.Vitamins, minerals, nutrition****Topic 19.Statistics,****Topic 20. Quality control in laboratory practice****Module 5****Topic 21.DNA, RNA, protein biosynthesis****Topic 22.Molecular biology techniques****Topic 23.Mutation, cell cycle****Topic 24.Advanced molecular biology techniques****Topic 25.Blood and blood components****Module 6****Topic 26.Immunology****Topic 27.Immunology continued****Topic 28. Clinical pathology, Microbiology and serology****Topic 29. Clinical Research****Topic 30. Research Methodology,**

Each topic will have

1. Objectives
2. Detailed text
3. Questions
4. Assignments at the end of each topic. Student should submit it in a time bound fashion, on line, and should be a pre-requisite for appearing before the Module examination.

Contact Programme

It is mandatory for completion of the course

Attendance is compulsory for all days of contacts assigned

Evaluation is done at the end of the programme

Fee

Rs. 10,000 per Module, sent as DD to the Office of College

CONTENTS OF THE SYLABUS

(Each **Topic** will be prepared by qualified senior teachers)

Semester 1**Topic 1. Biomolecules**

Chemistry of carbohydrates: Monosaccharides, glucose, fructose, galactose and mannose. Reactions, reducing property, Isomers, Mutarotation, anomers and epimers. Amino sugars and deoxy sugars. Glycosidic bond, disaccharides, lactose, sucrose, maltose, polysaccharides homopolysaccharides and heteropolysaccharides, starch, glycogen, dextrans, glycosaminoglycans.

Chemistry of amino acids and proteins: Structure and properties of amino acids. Classification based on structure, ionic properties of amino acids, isoelectric pH, buffering action of amino acids and proteins. Structural organization of proteins – primary, secondary, tertiary and quaternary, forces involved in maintaining, Protein denaturation, coagulation, isoelectric precipitation using salt solutions, classification of proteins, Methods to determine the amino acid sequence of proteins, conjugated proteins, nucleoproteins, lipoproteins, immunoglobulins, glycoproteins. Structure – function relationship of proteins; protein-protein interaction, Prions, beta amyloid.

Chemistry of lipids: Classification, saturated and unsaturated fatty acids, triacylglycerol, PUFA, compound lipids, phospholipids Cholesterol, steroids, Ecosanoids.

Cell structure & functions , subcellular organelles, biomembrane, transport and ion channels; Cell fractionation, Activities of different fractions, Marker enzymes

Membranes: Chemical composition, structure, biological properties, functions, membrane transport mechanisms, ion channels, endocytosis, exocytosis, and defects.

Topic 2. Basic knowledge

Quantitative methods: Principles and applications of: Photometry, Spectrophotometry (UV, visible, IR), Fluorimetry, spectroscopy, turbidimetry, nephelometry, spectrofluorimetry, atomic emission, reflectometry, mass spectrometry, nuclear magnetic resonance.

Analytical Principles and Techniques

Separation techniques including gas and liquid chromatography, paper, thin layer, ion exchange, affinity, gel filtration, gas liquid, HPLC, electrophoresis and dialysis;

Blood: Collection, anticoagulants, preservatives, deproteinisation, Specimen collection, handling and storage. Effects of Collection and Storage of Specimens. Place and time of sample collection, preservation, influence of nutrition, drugs, posture, etc. Choice and correct use of anticoagulants; Care of the specimens, identification, transport, storage, influence of temperature, freezing/thawing

Topic 3. Enzymes

Classification, nomenclature, enzyme specificity, kinetics of enzyme activity, Reaction kinetics with regard to catalyzed reactions coenzymes and cofactors, regulation of enzyme activity, Isoenzymes and their significance with suitable examples, proenzymes, multienzyme complexes, tandem enzymes, covalent modification, active site of enzymes, methods of locating the amino acid residues in the active site, mechanism of enzyme action. Enzyme inhibitors: competitive, non-competitive, Allosteric, suicide. Enzyme kinetics, factors affecting enzyme activity, K_m value and significance, Purification of enzymes: characterization and criteria of purity. Purification of proteins.

Topic 4. Diagnostic enzymology, including markers

Diagnostic importance of enzymes : LDH and isoenzymes, CPK, AST, ALT, NTP, GGT, ALP, ACP, GPD, Amylase, Lipase, , Serum GGT, 5' nucleotidase, Cholinesterase

Tumour markers, Bone markers, Cardiac markers, liver markers

Topic 5. Metabolism of amino acids and proteins

Body amino acid pool, dynamic state of body proteins, inter-organ transport of amino acids, nitrogen balance, glucogenic and ketogenic amino acids. Transamination, decarboxylation, oxidative deamination, transdeamination, formation of ammonia. Urea cycle. Metabolism of glycine, compounds synthesized, creatine and creatinine, glutathione hyperglycinemias, Metabolism of sulphur containing aminoacids, methionine and cysteine, transsulphuration, transmethylation reactions, formation of taurine, PAPs, excretion of sulphur, homocystinuria, cystinuria and cystinosis. Phenyl alanine and tyrosine – metabolism, phenyl ketonuria and alkaptonuria, synthesis of thyroid hormones , synthesis and catabolism of catecholamines, albinism, tyrosinemia. Tryptophan – formation of NAD, serotonin and 5 HIAA, xanthurenic acid, melatonin, formation of indican, Hartnup's disease. Branched chain amino acids, maple syrup urine disease, Glutamic acid, glutamine, GABA, aspartic acid, , serine, (metabolic role and compounds synthesized using these amino acids), polyamines. Inborn errors of metabolism, urine screening

Module 2**Topic 6. Estimation of proteins, electrophoresis**

Procedures and application of: Electrophoresis, paper, polyacrylamide gel, agarose gel, and cellulose acetate. Ultra centrifugation, ultrafiltration. Estimation of amino acid, Ninhydrin (colorimetric) method. Protein estimation by Lowry's method. Estimation of proteins by Folin's method Estimation of protein by dye binding method. Estimation of nitrogen content of protein by Kjeldahl method. Absorption spectra of proteins.

Estimation of Albumin, total proteins and Albumin globulin ratio in serum. Biuret and dye binding method.

Topic 7. Metabolism of carbohydrates, TCA cycle

Methods of investigation of metabolic pathways. Primary secondary and tertiary metabolisms.

Carbohydrate metabolism: Digestion and absorption of carbohydrates, Glycolysis: Reactions, regulation in brief, energetics, Rapaport leuberling cycle, fate of pyruvate in aerobic and anaerobic conditions, PDH reaction. Gluconeogenesis, key enzymes, regulation and significance, Cori's cycle. Glycogen synthesis and degradation, regulation, inborn errors associated. HMP shunt pathway, NADPH generation, transketolase reaction, G6PD deficiency, metabolic importance. Metabolism of galactose, fructose, polyol and uronic acid pathways – inborn errors associated with carbohydrate metabolisms. Glycogen storage diseases, fructosuria, fructose intolerance, pentosuria, galactosuria.

Blood glucose regulation – hormones influencing carbohydrate utilization, insulin, glucagon, glucocorticoids, epinephrine, growth hormone.

Diabetes mellitus – Aetiology, biochemical abnormalities, symptoms and complications. Clinical features and laboratory findings in insulin resistance, Type 1, Type 2 and gestational diabetes mellitus; diagnostic and monitoring criteria for diabetes; investigation of hypoglycemic syndromes Glucose tolerance test procedures and interpretation; in pregnancy. Ketosis and lactic acidosis. Differential diagnosis of coma; hyperosmolar coma. Hemoglobin A1c or glycohemoglobin; fructosamines; C-peptide. Insulin tolerance test; glucagon and somatostatin. Use and dangers of provocative tests, e.g. tolbutamide and glucagon. Assay of insulin, proinsulin and insulin antibodies. Albuminuria Antibodies (anti-GAD, Anti-insulin, etc.).

Topic 8. Carbohydrates continued

Regulation and integration of metabolisms: TCA cycle, integration of metabolisms, common terminal pathways, Interrelation of carbohydrate, lipids, and amino acid metabolism regulation, Anaplerotic reactions, amphibolic role of TCA cycle amphibolic role. Hormonal regulation of

metabolic pathways, Cori's cycle, Alanine cycle, adaptation during starvation.

Bioenergetics and biological oxidation: Concept of free energy change, high energy compounds, Electron transport chain - components and sites of ATP synthesis ATP generation, ATP synthase complex and mechanism of oxidative phosphorylation redox potential, oxidative phosphorylation, inhibitors, uncouplers, ionophores. a Reactions, regulations and energetics

Estimation of glucose by Glucose oxidase method.

Estimation of glucose by ortho toluidine method.

Estimation of glucose by Nelson-Somogyi method.

Glucose tolerance test

Glycated hemoglobin, by affinity chromatography.

Topic 9. Liver Function Tests

Digestive enzymes in the various sections of the digestive system including the exocrine functions of the liver and pancreas, Hydrochloric acid, bicarbonate and bile secretion, Gastrointestinal hormones, Malabsorption including vitamin malabsorption, Exocrine functions of the pancreas, Acute pancreatitis, Chronic pancreatitis

Liver and biliary tract, Normal and disturbed functions of liver; metabolism; synthesis, biotransformation; excretion, Enterohepatic circulation; metabolism of bilirubin and bile acids, Hepatitis, cirrhosis, cholestasis, cytolysis

Liver function tests: Plasma proteins, albumin globulin ratio, functions of albumin, jaundice, types, Hb metabolism: Synthesis and degradation of hemoglobin, hemoglobinopathies, thalasseмии, porphyrias, heme synthesis and degradation, bilirubin metabolism, jaundices, laboratory findings in jaundices. Serum bilirubin estimation. Porphyrins and bile pigments; Porphyrins, lead intoxication and hyperbilirubinaemia, Electrophoresis, normal and abnormal hemoglobins.

Important plasma proteins (albumin, immunoglobulin, haptoglobin, transferrin, C-reactive protein, etc.) Dysproteinaemia, monoclonal components, Tumour-associated proteins

Topic 10. Kidney function tests

Functional anatomy of kidney, Structure & Function of Kidney, renal blood flow and its peculiarities. Glomerular filtration rate - Definition, Measurement & Factors affecting GFR. Glomerular functions – Reabsorption, Secretion, Acidification & Concentration and abnormalities. Kidneys and urinary tract Excretory substances in the plasma and urine. Glomerular filtration rate and clearance. Activity and effects of diuretics; free water clearance; alkalosis, Proteinuria, Acute and chronic renal insufficiency, nephritis, nephrotic syndrome, clearance tests, urea, creatinine estimations
 Urine analysis: Normal and abnormal constituents of urine (), urine preservatives, 24-hr assays, protein, calcium, phosphorus, urea, creatine, creatinine

Kidney function tests, blood urea, creatinine, creatinine, uric acid, clearance tests, Cystatin C

Protein estimation in urine and microproteinuria.

Physical and chemical analysis of urine, pH, specific gravity, reducing substances, protein, ketone bodies, blood, bile salts, bile pigments, Urinary aminogram, Urine urea, creatine, creatinine, uric acid, calcium, phosphorus

Molecule 3

Topic 11 : Hemoglobin metabolism

Topic 12. Metabolism of lipids

Digestion and absorption, transport and storage, fatty acid synthesis, fatty acid oxidation pathways. Beta oxidation of fatty acids, role of carnitine, regulation and energetics. Biosynthesis of phospholipids, glycolipids, inborn errors of metabolism. Role of adipose tissue and liver in lipid metabolism. Hormonal regulation of lipid metabolism. Formation and utilization of ketone bodies, ketoacidosis in diabetes and starvation Inborn errors, Cholesterol – structure, synthesis, regulation, metabolic fate, bile acids and steroid from cholesterol, lipoproteins, apoproteins, functions, dyslipidemias, atherosclerosis, biochemical basis of management of hyperlipidemias
 Metabolism of adipose tissue, hormone sensitive lipase, action of hormones (insulin, glucagon, epinephrine & cortisol) liver adipose tissue axis, fatty liver, lipotropic factors, Polyunsaturated fatty acids. Prostaglandin synthesis and metabolism. Obesity. Eicosanoids – prostaglandins, thromboxins and leukotrienes, structure, formation and actions. Inborn errors: lipid storage diseases

Topic 13. Cholesterol, lipid profile

Transport of plasma lipids, lipoprotein metabolism, lipid profile and – diet, PUFA and dietary fiber, Hypolipidemic drugs. Lipid storage diseases. Electrophoresis, Lipoproteins. Serum triglycerides.

Topic 14. pH, acid base balance

Concept of pH and buffers. Henderson-Hasselbalch equation. Principles and procedures of determination of pH, Preparation of buffers, Acid base regulation, buffer capacity. Acid and Bases in the body, plasma buffers, respiratory and renal regulation of pH, Acidosis and alkalosis, major causes and compensatory mechanism, anion gap, assessment of acid base status. Fluid and electrolyte balance – distribution of body water and disorders Chloride, bicarbonate, pCO₂, Na, K estimations, ion selective procedures, flame photometry

Topic 15. Pharmacology, Toxicology, Therapeutic Drug Monitoring

Drug absorption, metabolism, excretion; hepatic enzyme induction. Pharmacokinetics, pharmacodynamics and pharmacogenetics. Monitoring therapeutic levels of drugs, concepts of half-life, volume of distribution, peak and trough concentrations, area under the curve (AUC) and dosing intervals, toxicity levels. Symptoms and signs of overdose, use of antidotes. Detecting and quantifying poisons: e.g. methanol, ethylene glycol, lead, monoxide, organophosphorous compounds (cholinesterase). Drugs of abuse and modalities of DAU testing.

Drug monitoring, Pharmacokinetics, pharmacodynamics and bioavailability of drugs, Therapeutic range, Individual determinations for the most important drugs: digoxin, theophylline, anticonvulsants, etc.

Toxicology: Biochemical and physiological actions and the screening procedure for detection of the following compounds: Organophosphorus insecticides, ethanol, methanol, barbiturates, copper, mercury, lead, arsenic, cyanide, mineral acids, formic, oxalic acids. Patho mechanisms of the most important types of poisoning. Individual determinations for the most important types of poisoning, e.g. ethyl alcohol, carbon monoxide, barbiturates, methaemoglobin, methyl alcohol, ethyl enegycol, benzene, toluene, etc. Cholinesterase in the case of organic phosphate Intoxications, Tests for drugs of abuse

Module 4

Topic 16. Endocrinology

Mechanisms of action of hormones, Hormonal regulation, hormone transport, hormone receptors, signal transduction, G-proteins, second messengers, cyclic AMP, cyclic GMP, calcium, Inositol triphosphate, diacyl glycerol. Mechanisms of transcription and translation need to be understood in order to explain disorders of signal transduction. These may be due to a defect in the ligand (1st messenger), the membrane receptor, the signal system, an abnormal 2nd messenger, the nuclear receptor. Examples of membrane receptors include: Disorders of insulin receptors (insulin resistance); growth factor receptors (stunted growth, cancer); LDL-receptors (familial hypercholesterolemia); dopamine receptors (Parkinson's disease, tardive dyskinesia, schizophrenia, idiopathic hyperprolactinemia); muscarinic, cholinergic and GABA receptors (Huntington's disease); adrenergic receptors (asthma); other receptors. Antibodies to receptors: Ab to insulin receptor (acanthosis nigricans, ataxia-telangiectasia). Ab to TSH receptor (Graves' disease); Ab to acetylcholine receptor (myasthenia gravis). Transducer, G-protein defects: inactivated (pseudohypoparathyroidism); activated (cholera). Examples of intracellular receptors: androgen receptors (testicular feminization syndrome, Reifenstein's syndrome); estrogen and progesterone receptors (mammary cancer); glucocorticoid receptors (primary cortisol insensitivity); aldosterone receptors (pseudo hypoaldosteronism); Vitamin D receptors (hereditary resistance to Vit. D), T3 receptors (resistance or toxicity).

hormonal regulation of gene expression, hormonal disorders,

Pituitary and hypothalamic hormones. metabolic regulation of hormones, biochemical investigation of endocrine disorders,

Control mechanisms - hypothalamic hormones - anterior and posterior pituitary hormones;

Thyroid hormones, iodine metabolism, Neonatal thyroid screening.

Adrenal and medullary hormones, adrenal function test. Use of stimulation and suppression tests in the assessment of function and/or differential diagnosis of primary and secondary endocrine disease.

- use of the ACTH, dexamethasone, water deprivation, and bolus tests;
- endocrine rhythms, responses to diet, exercise, glucose, insulin, pregnancy, posture

Hormones that regulate calcium metabolism. Parathyroid & Calcium metabolism. Clinical features and laboratory findings in disorders of calcium metabolism and the parathyroids. Significance of the concentration of calcium, inorganic phosphorus and magnesium in serum and urine. Effects and properties of parathyroid hormone, calcitonin and Vitamin D. Biochemical aspects of bone metabolism; tetany. Differential diagnosis of hyper- and hypocalcemia; parathormone assays in blood, PTHrP. Phosphate metabolism: causes and effects of Pi excess and deficiency. Disorders of cholecalciferol hydroxylation. Calcitonin in the treatment of Paget's disease. Bisphosphonates in the treatment of osteoporosis, osteogenesis imperfecta and fibrous dysplasia. Magnesium metabolism, causes and effects of Mg deficiency. Osteocalcin and pyridinoline crosslinks for follow-up. Osteoporosis.

Hormones of pancreas and gastro intestinal tract. Endocrine Pancreas, Local Hormones.

Biogenic amines, Metabolism, Catecholamines, serotonin and their breakdown products, Prostaglandins

Male Reproductive System hormones

Female reproductive system hormones, Pregnancy, perinatal laboratory analysis

Hormones of placenta, the pituitary-hypothalamus system

Hormone measurements for Differential diagnosis :

- acute and chronic lesions of the hypothalamus and pituitary;
- hypo- and hyperthyroidism, goitre, thyroiditis;
- pheochromocytoma;
- Addison's disease and Cushing's syndrome; adrenogenital syndromes;
- renin / angiotensin / aldosterone disorders;
- gonadal hypo- and hyperfunction, infertility, amenorrhea, hirsutism;
- feto-placental disorders, genetic defects;
- endocrine aspects of hypertension and edema;
- ectopic endocrine tumors.

Topic 17.RIA, ELISA, hormone estimations

Isotopes: Detection and measurement of radioactive isotopes. Principles and application of tracer techniques in biology; Radiation dosimetry. Application of isotopes in research and clinical biochemistry. Effect of

radiation on biological system; Autoradiography, Liquid Scintillation spectrometry. Conjugation with ALP or HRP. FITC conjugation of antibody

Topic 18. Vitamins, minerals, nutrition

Vitamins: Fat soluble and water soluble vitamins, chemistry, sources, requirements, absorption, metabolism, biochemical role, coenzyme forms, requirements, deficiency manifestations of the following

vitamins	A	D	E	K
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Biochemical role, co-enzyme function, sources, requirement and deficiency of Thiamine, riboflavin, niacin, biotin, pyridoxine, pantothenic acid, folic acid and one carbon groups and B12, ascorbic acid

Mineral metabolism: Macrominerals, Sources, requirement, absorption, biochemical role, deficiency and toxicity of the following minerals, Sodium, Potassium, Chloride, Magnesium, Calcium, Phosphorus; role of PTH, 1,25 DHCC and CT.

Trace elements, Iron, anemias, Copper, Manganese, Zinc, Cobalt, Molybdenum, Selenium, iodide, fluoride. Atomic absorption spectrophotometer

Serum calcium estimation. Serum inorganic phosphorus estimation. Serum iron and iron binding capacity.

Nutritional requirements: Energy requirements, balanced diet, nutritional disorders, starvation, PUFA, dietary fibres, cholesterol. Protein requirements: nitrogen balance, nutritive value, biological value, chemical score, protein energy malnutrition, Kwashiorkor and Marasmus.

Clinical features and laboratory findings in disorders of the plasma proteins; acute phase proteins. Serum protein and albumin, serum and urine protein electrophoresis, Causes of hypoalbuminemia; hypo- and hyperglobulinemias Alpha-1-antitrypsin deficiency. Positive and negative nitrogen balance Protein requirements: in malabsorption, post-operative, protein wasting diseases. Anorexia, total parenteral nutrition. Obesity, risks to health, treatment methods. Assessment of nutritional status.

Topic 19. Statistics,

Biostatistics: Methods of collecting data, tabulation and representation of data, sampling and sample design, types of classification, tabulation, diagrammatic representations, line diagram, bar diagram, pie diagram, histogram, frequency polygon, frequency curves, cumulative frequency curves. Measures of central tendency: Mean, mode, median, range, mean deviation, standard deviation and standard error. Correlation analysis and regression analysis, probability, analysis of variables. Analysis of variance, one-way classification and two-way classification.

Concepts of parametric and non-parametric statistics, probability and significance, standard deviation, confidence limits, Tests of significance, t-test, F-test, analysis of variance, Chi square, linear and other regression, difference plots, non-parametric testing. Sensitivity, specificity, predictive value and ROC curves; Odds ratios; assessing the efficiency of a test.

Cluster discriminant function and trend analysis; multiples of the median (MoMs).

Study of available free softwares

Topic 20: Quality control in laboratory practice

Laboratory management: Reference intervals and biological variability
Reference methods and statistical comparison of methods, Use of reference values: influences of age, sex, lifestyle, etc. and decision value limits

Genetic influences, environmental influences, age, sex, nutrition, season and time of day, influence of therapeutic agents. Predictive value of analytical methods, diagnostic sensitivity and specificity, Internal quality assurance and external quality assessment, Interfering factors

Precision, accuracy, specificity, sensitivity, percentage error, quality control, precautions in handling hazardous materials.

Calibration of volumetric and gravimetric equipment and apparatus, handling and disposal of infected, dangerous materials, accidents, safety measures, emergency treatment, automation in clinical biochemistry

Methods of generating requests for laboratory tests; requisition forms, computerized order entry, selective requests versus organ system profiles; screening procedures; function-tests; pre-arranged batteries of tests or algorithms to answer specific clinical problems.

Specimen collection, identification, transport, delivery, preparation and pr
Errors of laboratory instruments; standardization units (S.I. and conventional); internal quality control, external quality control; statutory proficiency testing programs; primary and secondary standards; reference materials (International reference materials) and reference methods; evaluation and comparisons of methods and instruments, including

precision, accuracy, functional sensitivity; intra-individual biological variations; total analytical error, out-of-laboratory equipment and device quality control.

Laboratory reporting systems: Ensuring that the information reaches the attending physician within a time-frame for appropriate action. Critical values. Directing attention to abnormal results when necessary; providing clinical interpretation when appropriate. Laboratory information systems (LIS), electronic data transfer and instrument interfacing; data-base structure and data-mining.

Preparation and maintenance of proper laboratory manuals. Accreditation requirements.

Biological variations: Understanding the effect of physiological variables diurnal and individual variations, rest, exercise, age, sex, fasting, and the effect that drugs may have on test results. Chemical interference. Understanding the effects of interference by drugs, haemoglobin, lipemia, icterus, heterophilic antibodies and other substances.

Principles of methods for reduction of data into forms suitable for electronic data handling systems (computerized accessioning functions, sample identification and tracking (e.g. bar code systems), result reporting, storage and retrieval, electronic data transfer). Data management: medical informatics, data processing and telecommunication, presentation and communication of results of investigation (choice of units, design and content of request and report form)

Use of computers in quality control and management; use of computers for calculating analytical results (eg. non-linear functions).

Laboratory information systems (LIS), Hospital information systems (HIS).

Security of data storage and transmission. Data base structures and data mining. Appropriate access control to patient information.

Maintenance of records: Procedure manuals, ward manuals, quality control programs, patient data retrieval.

Laboratory safety: Fire, chemical (WHMIS), radiation and infection control (body substance precautions), hazardous waste and transport of hazardous materials.

Indications for Clinical Chemistry Procedures: In the early detection of disease, In disease-related diagnosis, In organ-related diagnosis, In monitoring vital functions, In monitoring response to therapy, In the field of drug monitoring, Indications for subsequent specialist examinations, Indications for function tests

Professional ethics. Quality assurance; total quality management; development and monitoring of performance indicators. Quality assessment

Statistical applications in the clinical laboratory: biological variation, establishment of reference intervals, methods comparison

Laboratory safety: Handling of potentially infectious samples (HIV and hepatitis), handling of noxious chemicals and isotopes, mechanical and electrical safety, fire precautions, dealing with an accident

Semi auto methods/alternative methods that should be followed even with lab automation. (esp when workload not very high / instruments like nephelometer/ mass spec ,etc cant be afforded)

Lab accreditation processes (NABL, JCI, CAP)

Module 5

Topic 21. DNA, RNA, protein biosynthesis

Nucleic acids: Chemistry and structure of bases, nucleosides, nucleotides, RNA and DNA. Mitochondrial DNA. Properties of nucleic acids.

Purine and pyrimidine metabolisms: synthesis and degradation of bases and nucleotides, regulation, hyperuricemia, Gout, Salvage pathway, Leish-Nyhan syndrome. Pyrimidine – nucleotide synthesis, regulation, orotic aciduria, thymidilate synthase reaction , folic acid antagonists and nucleotide analogues as chemotherapeutic agent

DNA replication, eukaryotic genomic organization, coding and non-coding sequences, satellite DNA DNA ligases, Topoisomerases, Gyrase, Methylases, Nucleases, DNA damage and repair processes, amplification of genes, somatic recombination, Telomeres, Lysogeny and lytic cycle of bacteriophages, Bacterial transformation, Host cell restriction, Transduction, complementation. Molecular recombination inhibitors of replication, anticancer drugs.

Transcription: Details of transcription, RNA polymerase, transcriptional control of gene expression, Operon concept, Lac operon, Tryptophan operon, regulation in eukaryotes, gene amplification, promoters, enhancers, silencers, hormone responsive elements, transcriptional factors, post – transcriptional modifications of RNA, exons, introns, splicing, Ribozymes, inhibitors of transcription, reverse transcriptase.

Translation: Genetic code, tRNA, ribosomes, rRNA, mRNA, protein biosynthesis, post – translational modifications, inhibitors of translation, signal sequences, chaperones and heat shock proteins, protein targeting, anticancer drugs.

Protein degradation: Cathepsins, Ubiquitins, Proteosomes, Caspases.

Topic 22. Molecular biology techniques

DNA Techniques: sequence determination, PCR, Southern, Northern and Western blotting procedures. In-situ hybridization. Polymerase chain reaction. Restriction mapping and restriction fragment polymorphism. DNA finger printing. Human genome project.

Recombinant DNA technique, restriction endonucleases and RFLP. cloning strategy, vectors, plasmids, phages, cosmids, shuttle vectors, expression vectors, and restriction map.. Genomic library and cDNA library. Microinjection technique. Transgenic animals. Gene therapy.

Isolation of DNA from animal tissue., Isolation of plasmid / bacteriophage DNA., Restriction digestion and agarose gel electrophoresis. Southern blotting, Western blotting, polymerase chain reaction and applications , application of molecular biology in clinical situations

DNA probes; restriction fragment length polymorphism (RFLP); polymerase chain reaction (PCR); amplification of mRNA (B). Diagnosis of genetic diseases by molecular biology techniques (thalassemias, sickle cell diseases)

Topic 23. Mutation, cell cycle

Mutations: spontaneous, induced, point mutation, silent mutation, frame shift, physical and chemical mutagens, site directed mutagenesis, Ames' test, carcinogens, in-vitro mutagenesis. Viruses and carcinogenesis, Oncogenes, Tumour markers, Hemoglobinopathies, phenylketonuria, alkaptonuria, homocystinuria, Lesch-Nyhan syndrome, Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Hemophilia, prenatal diagnosis of genetic diseases,

Cell biology: Cell cycle, mitosis and meiosis, check points, regulation, Oncogenes, tumor suppressor genes, apoptosis, tumor markers. Cancers as examples of defective cellular differentiation; strategy of cancer detection by biochemical means; cancer markers, cancer screening, carcinoembryonic antigen (CEA); alpha-fetoprotein (AFP); human chorionic gonadotropin (hCG); total and free prostate specific antigen (PSA); CA 19-9, CA 125, other antigens.

Oncogenic enzymology: acid phosphatase (ACP); alkaline phosphatase (ALP); lactate dehydrogenase (LD); other enzymes. Other body fluid constituents of use in oncology. Hormone receptors and response to therapy. Primary neoplastic endocrinopathies; paraneoplastic syndromes; multiple endocrine neoplasia (MEN) syndromes type I and type II; syndromes due to

ectopic hormone synthesis. Cancer associated with familial and congenital disorders (breast and colon cancers). Oncogenomics: susceptibility genes.

Topic 24. Advanced molecular biology techniques

Chromosomes, structure, number, sex chromosomes. Human karyotyping. Methods of chromosome analysis, chromosome banding, FISH, flow cytometry.

Techniques for nucleic acid analysis: amplification, investigation of mutations and gene expression

Molecular biology of hereditary disorders, Molecular biology investigations of non-infectious diseases, Prenatal diagnosis of inborn errors of metabolism

Topic 25. Blood and blood components

Blood: Composition & Functions of Blood , Composition of plasma in health and diseases, normal values, TC, DC, ESR, HB, PCV, bleeding time, clotting time, prothrombin time, fibrinolysis

Erythrocytes: Morphology, Count, Function, Erythrocyte Sedimentation rate, Osmotic Fragility, Anticoagulants, antiplatelet drugs, fibrinolytic agents. Erythropoiesis, Anaemia, Polycythemia, Fate of RBC, Jaundice.

Diffusion of gases, O₂ transport, CO₂ transport. Regulation of respiration – Voluntary, Neural, Chemical. Abnormalities of Respiration Hypoxia, Cyanosis, Dyspnea, Asphyxia., High altitude, Anaemia, Haemoglobinopathy. Thalassaemia and related disorders; characterisation by DNA analyses

Blood groups and its importance, Blood transfusion.

Leucocytes - Morphology, Types, Properties & Functions, Leucopoiesis & Variation. Thrombocytes- Morphology, Count, Function, Variation. Leucocytosis, Leukemias.

Thrombopoiesis and Hemostasis. Coagulation & its disorders., Thrombocythaemia/ thrombocytopenia, Hereditary and acquired disorders, bleeding and thrombosis, Mode of action of anticoagulants, fibrinolytics, and aggregation inhibitors Characterisation of normal and disturbed clotting functions, Investigation of platelet function. Influence on platelet aggregation by ADP-adrenaline, collagen, ADP, ATP. Spontaneous

aggregation. Clot retraction. Platelet factor III determination, glass pearl test
 Use of chromogenic substrates for the determination of coagulation factors
 Detection of circulating inhibitors, thrombo test diluted curve, cephalin dilution curve, Protein S, protein C, prekallikrein, high molecular kininogen determination, plasminogen, antiplasmin, plasminogen activators
 Immunohaematology and blood banking, Typing of irregular (auto) antibodies; determination of antibody titre, Extended blood group typing (beyond ABO and Rhesus D)
 Organisation of blood banking, Typing of B and T lymphocytes, Platelet antibodies, Typing of leucocytes and tissue antigens
 Plasma proteins, Cerebrospinal fluid, other body cavity fluids Acute phase proteins; Alpha-1 anti-trypsin, Alpha-2 macro-globulin, Haptoglobin, Ceruloplasmin.
 Cerebrospinal fluid (CSF), CSF synthesis and circulation, Composition of CSF in comparison to serum, CSF cell counting and differentiation, Hereditary and acquired disorders of CSF homeostasis

Semester 6

Topic 26. Immunology

Basic concepts of immunology: Innate and acquired immunity, inflammation, inflammatory cells, mediators, inflammatory responses, Cells and organs of immune system and evolution of immunity. Functions of the humoral and cellular immune systems and their regulation; cytokines; inflammation; acute phase proteins, Surface antigens, B cells, T cells, helper T cells, Cytotoxic T cells, NK cells, macrophages, Interleukins, Cytokines, Antigens, antigen processing, MHC molecules, receptors, Clonal selection.

Chemistry of immunoglobulins, classes and subclasses, cryoglobulins, immunoglobulins genes, organization and expression, generation of antibody diversity, class switching, primary and secondary immune response, regulation of immune response.

Complement system, complement receptors, activation pathways, control mechanisms, role in inflammation, kinin cascade, kinins in diseases.

Vaccines: Immunity against bacteria, virus, fungi and parasites, advances in the development of vaccines, pertussis, cholera, malaria, hepatitis, polio, antitumor adjuvants.

Auto immunity: Allergy & inflammation, Histamine, 5HT, Prostaglandins, PAF, Bradykinin, Adenosine- role in allergy & inflammation, pharmacological manipulation, Non-steroidal anti-inflammatory drugs,

Pathogenesis of asthma & principles of therapy. Anaphylaxis, Delayed hypersensitivity, contact dermatitis, skin tests, multiple sclerosis, thyroiditis, granulomatous reactions (tuberculosis and leprosy), drug allergy (penicillin), assays for T cells and B cells, HLA antigens, graft-host relationship. Immunodeficiency including AIDs, tumour and transplantation., Immunoglobulin deficiency and overproduction, monoclonal and polyclonal immunopathies Major histocompatibility complex

Topic 27. Immunology continued

Immuno-hematologic diseases: Transfusion reaction, erythroblastosis, fetalis, warm and cold antibody diseases, drug and hemolytic diseases, agranulocytosis, thrombocytopenic purpura

Immune complexes: Arthus reaction, serum sickness, glomerulonephritis, skin diseases, evaluation of circulating immune complexes

Connective tissue diseases: Polyarteritis, SLE, rheumatic fever, rheumatoid arthritis.

Immunological tests: agglutination tests, direct and indirect, hemagglutination, Latex agglutination and hemagglutination inhibition, complement fixation test, immunodiffusion, radial immunodiffusion, immunoelectrophoresis, Radio Immuno Assay (RIA), Enzyme Linked Immunosorbent Assay (ELISA), Western blotting, Nephelometry, immunofluorescence, Florescent In Situ Hybridization (FISH) techniques, monoclonal antibodies, Hybridoma technology flow cytometry, fluorescent activated cell sorter and clinical application, lymphocyte activation, mixed lymphocyte culture, neutrophil function tests, histocompatibility testing. Immunochemical techniques: Immunochemical protein analysis, immunofixation, immunonephelometry and turbidimetry Immunological and other binding analysis using different labels; homogeneous and non-homogeneous immunoassays.

Topic 28. Clinical pathology, Microbiology and serology

Cell and particle counting methods

Principles of sterilization. Common microbial organisms.

Microbial Diagnostic procedures, Specimen selection and collection (blood, urine, sputum, faeces, others), Specimen processing: smears, staining, cultures, susceptibility testing

Usual techniques for microbe and virus identification

Molecular biology techniques for characterization of microbes and viral agents

Bacteria: *Neisseria gonorrhoeae* and *N. meningitidis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *S. agalactiae* and *S. pneumoniae*, *Escherichia coli*, *Salmonella typhi* and *S. typhimurium*, *Shigella* spp, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Clostridium perfringens*, *C. tetani*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Treponema pallidum*, *Chlamydia trachomatis* etc.

Viruses: cytomegalovirus and others: influenza, poliomyelitis, hepatitis A, B and C, human immunodeficiency (HIV), herpes etc.

Diseases and syndromes. Epidemiology, main clinical signs, bases for biological diagnosis, treatment, Meningitis, Septicaemia, Urinary and vaginal infections, Bacterial and viral diarrheas, Respiratory infections, Sexually transmitted diseases, Hepatic virus infections, Cytomegalovirus infections

Parasitology: Epidemiology, main clinical signs, diagnosis and treatment of: Amoebiasis: *Entamoeba histolytica*, Giardiasis, cryptosporidiosis and uro-genital trichomoniasis, Malaria, Toxoplasmosis, Intestinal, hepatic and urinary helminthiasis:, strongyloidiasis,, ancylostomiasis, enterobiasis, ascariasis, schistosomiasis (*Schistosoma mansoni* and *S. haematobium*) fascioliasis (*Fasciola hepatica*) taeniasis (*Taenia saginata*), Leishmaniasis, Filariasis

Fungal infections (*Candida albicans*, *Cryptococcus neoformans*), *Aspergillus* infections (*Aspergillus fumigatus*), Dermatophyte infections

Antibiotics and chemotherapeutic agents: Inhibition of cell-wall synthesis, nucleic acid and protein synthesis, specific enzyme system, Damage to cytoplasmic membrane. Antifungal antibiotics, Antiviral chemotherapeutic agents, Drug resistance, Assay of antibiotics. Susceptibility to chemotherapeutic agents. Antibiotic sensitivity test

Topic 29. Clinical Research

Pre clinical Models; (Drug discovery and development, including animal studies, tissue culture studies, safety, efficacy) ICH Guidelines on Preclinical Safety, Assessment of pharmacokinetics in early phase drug evaluation, Metabolism studies: in vitro and in vivo tests,

Pharmacogenomics in drug metabolising enzymes, Bioanalytical techniques: HPLC, MS, GC, RIA

Pharmacodynamics, Measuring drug activity in humans, Clinical Studies – Typical Designs, General toxicity testing, Acute, Subacute and Chronic Toxicity Studies, Testing for Carcinogenicity, Safety and Efficacy Studies

Human Ethics Introduction to the Ethics in Research, ICMR guidelines on Clinical Research. , Patient's rights and safety, conduct of illegal / unethical trials. The Nuremberg Code of 1946, Thalidomide Disaster of 1962, Declaration of Helsinki, 1964, ICH-Good Clinical Practice of 1997, Ethical Guidelines for Biomedical Research on Human Subjects, ICMR, 2000, Good Clinical Practices, 2001(Indian GCP)

General Principles

Principles of voluntariness, informed consent and community agreement, Principles of non-exploitation, Principles of privacy and confidentiality, Principles of accountability and transparency, Principles of totality of responsibility, Research in special groups - pregnant or nursing women, children, vulnerable groups, Compensation for participation, compensation for Accidental injury

Ethical Review Procedures; Independent Ethics Committee: Basic responsibility, Composition, Standard Operating Procedures, Review Procedures, Submission of application, Decision making process, Interim Review, Record keeping, Special consideration

Animal ethics: Context of animal research-past and present, The capacity of animals to experience pain, distress and suffering, Animal rights, Moral principles relating to animal experimentation, Institutional Biosafety Committee –Constitution, Functions & Activity

Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA) Guidelines, Scientific methods of housing and management of lab animals, sacrificing animals after experimentation following CPCSEA guidelines, Record Keeping, SOPs, Anaesthesia, Euthanasia – guidelines

Phases, Pre clinical studies, Phase 0, Phase I, Phase II, Phase III, Phase IV, single blind and double blind studies, randomisation procedures

International Conference of Harmonization (ICH), ICH good clinical practice consolidated guideline, ICH clinical safety data guideline, ICH clinical safety data guideline

Source documents: - preparation, processing, archiving, review and completion of source documents according to required guidelines and standards, Subject information sheet, Informed consent form , Maintenance

of Site Files and other study related documents and equipments, Counsels study subjects and give instruction as per study protocol

Topic 30. Research Methodology

Planning, Review of literature, basic principles, Animal ethics committee
How to write an article for any international journal (in depth analysis of planning, materials, methods) etc

Basic principles of Management; Financial Management aspects which are essentially required for development of the laboratory

CONTACT MODULES

Contact classes should cover the following techniques

Spectrophotometric Techniques: Molar absorptivity, reflectance, absorbance, transmittance; fluorometry, fluorescence, nephelometry, chemiluminescence and turbidimetry.

Ion specific electrodes: electrolytes and other applications.

Blood gas apparatus and co-oximeters

Automated and semi-automated analysers for general chemistry, automatic sampling and pipetting devices, Random access and batch analysers

Osmometers.

Centrifuges, water baths, balances, microscopes, pH meters.

Water quality requirements; water purification systems, stills, de-ionizers, reverse osmosis; methods of checking the quality of water.

Small instruments for point-of-care testing.

Flame photometers

Freeze drying; dialysis; concentration, desalting, ultra-filtration;

Calibration techniques.

Enzymatic Techniques: Enzyme and isoenzyme measurement methods (fixed incubation and kinetic methods); standardization and optimization of methods; stability of enzymes. Immobilized enzyme assays (biosensors).

Protein estimation by Lowry's method., Estimation of proteins by Folin's method , Estimation of protein by dye binding method.

Estimation of Albumin, total proteins and Albumin globulin ratio in serum.

electrophoresis; monoclonal gammopathies; immunoglobulins (IgG, IgA, IgM), complement, urinary microalbumin, C-reactive protein.

Serum Alkaline phosphatase, AST, ALT, Total LDH and iso-enzymes., Serum GGT. acetylcholinesterase, angiotensin converting enzyme (ACE),

pseudocholinesterase, glucose-6-phosphate dehydrogenase (GPD), amylase isoenzymes, macroamylases

Isoenzymes (CK, LD, ALP).

Prostate specific antigen (PSA): total, free, complexed. Carcinoembryonic antigen (CEA-125); alpha-fetoprotein (AFP); chorionic gonadotropin (hCG).

Estimation of glucose by Glucose oxidase method, Glucose tolerance test, Glycated hemoglobin by affinity chromatography. Glycated serum proteins (fructosamines).

Ketones - Blood and urine. Lactate.

Serum bilirubin estimation, total, conjugated (direct),

Normal and abnormal hemoglobins.

Blood gases and pH, carboxyhemoglobin, CO, Met Hb, O₂saturation

Hematologic Disorders, Heme and Bile pigments.

General morphology of blood cells and blood cell counting

Determination of erythrocytes sedimentation rate; determination of haemoglobin concentration, haematocrite cell counts and knowledge of haematological parameters (MCV, MCH, MCHC, RDW)

Preparation and staining of blood smears, with microscopical evaluation

Measurement of Hb, detection of abnormal forms and hemolytic states.

Measurement of porphobilinogen, uro- and coproporphyrins, delta-aminolevulinic acid (ALA) and ALA dehydratase. Spectroscopy of the heme pigments, hemoglobinemia and hemoglobinuria.

Serum iron, iron-binding capacity, transferrin and ferritin.

Haptoglobin, hemopexin, myoglobinemia and myoglobinuria.

Anemias, hemolysis, the hemoglobinopathies. thalassemias

Bleeding, clotting, and transfusion.

White blood cells, Platelet biochemistry .

Flow cytometry and leucocyte sub-grouping

Coagulation tests, Determination of coagulation factors. Investigation of fibrinolysis, Determination of antithrombin III and heparin, Immunological determination of coagulation factors (factor deficiency, increased fibrinolytic activity)

Blood group typing, ABO and Rh(D); Detection of irregular antibodies, Cross matching of blood samples for transfusion, Indirect antiglobin test, direct antiglobin test, Blood group ABO antigens and other antigen systems, types of transfusion reactions

Detection and measurement of variant and minor (Hb A₂ and HbF)

Leukaemias, lymphomas, polycythemias

Cardiac Markers: CK-2 (CKMB), CK isoforms, troponins I&T, hsCRP, myoglobin

Quality control, internal and external, analysis of variance

Accuracy, precision, sensitivity and specificity.

Biuret and dye binding method.

Serum calcium estimation, Serum inorganic phosphorus.

Blood urea estimation, Serum creatinine, Serum uric acid

Lipids and Atherosclerosis (Homocysteinuria, hsCRP, lipids, lipoproteins and apoproteins in assessing risk and monitoring therapy), cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, apolipoproteins A and B, lipoprotein(a); apolipoprotein E, Lipid profile

ELISA: Tumor markers, T3, T4, TSH.

Western blotting.

Immuno-electrophoresis, immunoblotting and immunofixation; Immunoassays: isotopic and non-isotopic, competitive, non-competitive or immunometric, liquid or solid-phase. Enzyme-linked, immunoassays, nephelometric, turbidimetric and fluorometric immunoassays.

Chromatographic Techniques: Thin-layer, ion exchange resins, molecular sieves, affinity chromatography. Direct and reverse phase liquid chromatography, high pressure liquid chromatography; solid phase extraction techniques. Gas chromatography with FID, NPD and mass detection

Electrophoretic Techniques: Electrophoresis of serum proteins, cellulose film electrophoresis, polyacrylamide, capillary zone electrophoresis, isotachopheresis; isoelectric focusing,

Electrophoresis, Lipoproteins.

Trace metal analysis: Atomic absorption spectroscopy.

Drug analysis:

acetaminophen, aminoglycosides, phenobarbital, phenytoin, salicylates, carbamazepine, ethanol, theophylline, digoxin, lithium, valproic acid, amphetamines, cocaine, opiates, cannabinoids, PCP, barbiturates, benzodiazepines, tricyclics. quinidine, methanol, isopropyl alcohol, ethylene glycol, amphetamine, phenothiazines, diazepam, chlordiazepoxide; ethosuximide, primidone, lidocaine, cocaine, cyclosporin, tacrolimus.

Hormone tests:

hCG screen (pregnancy test) and quantitative hCG, thyroglobulin; cAMP, reverse T3, TSH-receptor antibody, calcitonin; thyroxin (T4) and free

thyroxin, thyrotropin (TSH), triiodothyronine (Total T3) and free T3, anti-TPO, anti-TG, thyroglobulin, cortisol (plasma and urinary free), estradiol, testosterone (total and free), dehydroepiandrosterone sulphate (DHEA-S), estrogen and progesterone receptors; progesterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), growth hormone (hGH), somatostatins; somatomedins; IGF-1 and IGF-BP3, insulin tolerance test; insulin, PTH (intact). 17-hydroxyprogesterone, 11-deoxycortisol, corticotropin (ACTH), aldosterone, gastrin, renin, C-peptide, glucagon; sex hormone binding globulin (SHBG), androstenedione, unconjugated estriol. prostaglandins; endorphins; antidiuretic hormone (ADH), atrial natriuretic peptide

Diagnostic Procedures: Adrenocorticotropin (ACTH) or Cortrosyn (synthetic ACTH) stimulation tests for adrenal insufficiency, saline infusion test for hyperaldosteronism, congenital adrenal hyperplasia or hirsutism. Dexamethasone suppression test for Cushing's syndrome, Clonidine or L-DOPA stimulation tests for growth hormone deficiency, Metanephrines, catecholamines, VMA.

Iron - serum, iron binding capacity, iron saturation, transferrin, ferritin.

Spinal fluid - glucose, protein, oligoclonal bands.

Amniotic fluid: bilirubin; alpha-fetoprotein; fetal lung maturity testing.

Ceruloplasmin.

Tumor markers: PTHrp, NSE, Her2Neu.

Acid Base and Respiratory Function

Clinical features and laboratory findings in metabolic and respiratory acidosis and alkalosis.

Flame photometry, Sodium and Potassium.

Plasma chloride, bicarbonate, pH and pCO₂ and pO₂

Role of hemoglobin, oxygen and carbon dioxide transport, carbon monoxide, etc. Control

of respiration; chronic respiratory failure

Disorders of Kidney and Urinary Tract

Clinical features and laboratory findings in acute glomerulonephritis, nephrotic syndrome, acute tubular necrosis, renal tubular acidosis, chronic renal failure. Normal and abnormal urine composition, including abnormal pigments. Urinalysis (including microscopy). Physical and chemical analysis of urine, pH, specific gravity, reducing substances, protein, ketone bodies, blood, bile salts, bile pigments, Urine urea, creatine, creatinine, uric acid, calcium, phosphorus, Creatinine clearance, Urea clearance., Protein

estimation in urine and microproteinuria. Urinalysis, stick tests, microscopic analysis.

Clearance tests: measurement of glomerular filtration rate; creatinine clearance; cystatin measurement, phosphate clearance(TmP/GFR).

Plasma creatinine, urea (blood urea nitrogen). Proteinuria. Urine anion gap.

Differential protein clearance, inulin clearance., Measurement of renal plasma flow., Tubular function tests: Concentration tests, Ammonium chloride loading test. Bicarbonate loading test., Amino acid chromatography; Renal calculi. Transplant biochemistry and immunology, Endocrine function of the kidney. Nephrotoxic drugs.

Hepatobiliary Disorders.

Clinical features and laboratory findings in neonatal jaundice, acute and chronic liver disease, intra- and extrahepatic cholestasis, hepatic and biliary malignancies. Metabolic functions of the liver. Monitoring liver disease. Bilirubin, conjugated bilirubin, delta-bilirubin, urobilinogen. Alkaline phosphatase, the aminotransferases, gammaglutamyl transferase. Differential diagnosis of diseases causing jaundice. Alcohol and liver disease. Viruses in acute and chronic liver disease; hepatitis A,B,C,D,E, F, G. Complications of chronic liver disease, bleeding, ascites, gall stones. Serum proteins, alpha-fetoprotein, immunoglobulins in liver disease. Prothrombin and vitamin K. Bile acids and liver disease. Blood ammonia, hepatic coma. Hepatotoxic drugs.

Familiarise with the following instruments

Capillary zone electrophoresis and Immuno-fixation electrophoresis. Ultracentrifuge. Tandem Mass spectrometers. Isotope ratio mass spectrometers, Biosensors. Infra-red spectrophotometers. Amino-acid analyzer. Preparative and high-voltage electrophoresis. Fluorescent in situ Hybridization (FISH). Proteomics, 2D electrophoresis, protein arrays.

Structure, synthesis, and function of immunoglobulins. CD4 / CD8 cells.

Disorders of the immunoglobulins: IgA, IgG, IgM; Quantitative determination of immunoglobulins and complement factors Monoclonal gamopathies, multiple myeloma, Bence-Jones proteinuria, cryoglobulins, hypo- and agammaglobulinemias: Immunoglobulin IgD, IgE, allergen specific IgEs; IgG subgroup quantification. Radial immunodiffusion.

Hemagglutination, Latex agglutination, Complement fixation test

Complement system, HLA system. Monoclonal Antibodies. Transplant biochemistry.

Antibodies: anti-ds-DNA; anti-nuclear antibodies by immunofluorescence, specific antinuclear antibodies (SS-A, SS-B, Sm, RNP); anti-TPO (anti-microsomal) and antithyroglobulin; anti-glomerular basement membrane; anti-insulin; anti-smooth muscle, anti-neutrophil cytoplasmic antibodies, anti-cardiolipin antibodies, antitransglutaminase and antigliadin antibodies.

Restriction digestion and agarose gel electrophoresis.

Southern blotting.

Polymerase chain reaction cyclers and other amplification techniques.

Molecular Diagnostics: Principles and methods of DNA and RNA isolation, purification, polymerase chain reaction (PCR). DNA probes (radioactive and non-radioactive labels), hybridization, restriction fragment length polymorphism (RFLP), Blotting techniques, DNA chips

Methods of genomic analysis particularly microarray methodology including DNA/RNA microarray, Protein microarray and Antibody microarray

TEXT BOOKS AND REFERENCES

Textbook of Biochemistry for Medical Students: D.M. Vasudevan and Sreekumari, S, 6th Edition, 2010, Jaypee Brothers Medical Publishers, New Delhi.

Concise Medical Biochemistry, 3rd edition, by Sucheta Dandekar

Biochemistry: Satyanarayana and Chakrapani; Books and Allied P Ltd.

Textbook of Biochemistry: Dinesh Puri

Harper's Biochemistry, Murray et al, Lange Medical Book, Prentice Hall.

Lippincott's illustrated review in biochemistry; Lippincott, Raven publishers.

Teitz Fundamentals of Clinical Biochemistry, WB Saunders's company,

Textbook of Biochemistry with clinical correlations: Thomas M. Devlin, Wiley Medical publishers, New York.

Molecular Medicine, an introductory text for students: RJ Trent, Churchill Livingstone

Biochemistry: A Case-Oriented Approach. Montgomery, R. *et al.* Mosby,

Clinical Laboratory Medicine: Clinical Application of Laboratory Data. Ravel, R. (Ed.), Year Book Med. Publ.

Clinical Chemistry: Theory, Analysis and Correlation. Kaplan, L.A. and Pesce, A.J., Mosby.

Clinical Diagnosis and Management by Laboratory Methods. Henry, J.B., Saunders.

Lecture Notes on Clinical Chemistry. Whitby, L.G., Percy-Robb, I.W., and Smith, A.F., Blackwell.

Cases in Chemical Pathology: A Diagnostic Approach. Walmsley, R.N., Watkinson, L.R., Koay, E.S.C., World Scientific

Fundamentals of urine and body fluid analysis, Nancy A Brunzel, WB Saunders Company

An introduction to practical Biochemistry: Plummer DT, Mc Graw Hill International Book Co

Toxicology, the basic signs of poisons, Cassertt and Doulla

Textbook of Medical Laboratory Technology: Praful B Godkar, Bhalani Publishers, Bombay.

Clinical Physiology of Acid-Base and Electrolyte Disorders. Rose, D.B., 4th ed. McGraw Hill.

Review of Medical Physiology. Ganong, W.F., Lange.

The Metabolic Basis of Inherited Disease. Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., 7th ed. McGraw Hill.

Williams Textbook of Endocrinology. Wilson, J.D. and Foster, D.W., Saunders,.

Advances in Clinical Chemistry. Latner, A.L. and Schwartz, M.K. (Eds.). Academic 1998. Annual volumes.

Annual Review of Biochemistry series.

Clinical Case Material for Teaching Clinical Chemistry and Laboratory Medicine. Allen LC, Dominiczak MH, Pulkki K, Pazzagli M. Clin Chem Lab Med 2001;39:875–889.

CLINICAL CASES ON THE WORLD WIDE WEB

<http://www.hmc.psu.edu/endo/clincorr/clincor1.htm>

<http://www.hmc.psu.edu/endo/clincorr/clincor2.htm>

<http://www.embbs.com/aem/aemweb.html> Clinical Pearls

<http://www.embbs.com/tox/tox.html> Toxicology Corner

<http://www.embbs.com/ccs/ccs.html> Challenging Clinical Cases

<http://www.ec.hscsy.edu/path/endo99/cvframe.htm>

http://pathreports.upstate.edu:81/lung_cases/frame.htm

<http://pathreports.upstate.edu:81/anemia/frame.htm>
<http://pathreports.upstate.edu:81/leuko/frame.htm>
http://pathreports.upstate.edu:81/gen_path/frame.htm
http://pathreports.upstate.edu:81/cardiac_cases/frame.htm
http://pathreports.upstate.edu:81/neuro_cases/frame.htm
http://www.mcl.tulane.edu/classware/pathology/medical_pathology/endocrine_cases/casesTop.html
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<http://www.uchsc.edu/sm/pmb/medrounds/index.html>
<http://www.vh.org/Providers/Textbooks/ElectricGiNucs/Text/CaseStudies.html>
<http://www.vh.org/Providers/TeachingFiles/PulmonaryCoreCurric/PulmCoreCurric.html>
<http://www.leeds.ac.uk/medicine/teaching/clinsci/lscases/lscindx.htm>
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JOURNALS, recommended

American Journal of Clinical Pathology
 Annals of Clinical Biochemistry
 British Medical Journal
 Clinical Biochemistry
 Clinica Chimica Acta
 Clinical Chemistry
 Clinical Chemistry and Laboratory Medicine
 Clinics in Laboratory Medicine
 Critical Review of Clinical Laboratory Sciences
 Current Clinical Chemistry
 Endocrinology
 Indian Journal of Clinical Biochemistry
 Indian Journal of Biochemistry and Biophysics
 Journal of Clinical Chemistry and Clinical Biochemistry
 Journal of Clinical Endocrinology and Metabolism
 Journal of Clinical and Laboratory Medicine
 Journal of Clinical Pathology

Lancet

Nature

New England Journal of Medicine

Scandinavian Journal of Clinical and Laboratory Investigation

Science.