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SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLGOY

UNIT – I - BASIC CONCEPTS OF CLINICAL BIOCHEMISTRY - SBC3104

UNIT 1 BASIC CONCEPTS OF CLINICAL BIOCHEMISTRY

Organization of clinical laboratory, Introduction to instrumentation and automation in clinical biochemistry laboratories, safety regulations and first aid.Sample collection and processing, Transport of specimens, concepts of accuracy, precision, test sensitivity, test specificity in the quantitative assessment of test performance

Organization of Laboratory:

Biochemistry: Biochemistry is a basic science which deals with chemical nature and chemical behaviour of living matter and with the reactions and processes they undergo. Biochemistry involves the study of: Chemical constituents of living matter. Chemical changes which occur in the organism during digestion, absorption and excretion. Chemical changes which occur during growth and multiplication of the organism. Transformation of one form of chemical constituent to the other. Energy changes involved in such transformation. Note:- The term "Biochemistry" was first introduced by German chemist Carl Neuberg in 1903 from Greek word "bios" means "life".

Clinical Biochemistry: It is mainly deals with the biochemical aspects that are involved in several conditions. The results of qualitative and quantitative analysis of body fluids assist the clinicians in the diagnosis, treatment and prevention of the disease and drug monitoring, tissue and organ transplantation, forensic investigations and so on. Various biological fluids subjected to chemical tests and assays include blood, plasma, serum, urine, cerebrospinal fluid (CSF), ascetic fluid, pleural fluid, faeces, calculi and tissues. Note:- Modern day medical practice is highly dependent on the laboratory analysis of body fluids, especially the blood. The disease manifestations are reflected in the composition of blood and other tissues. Hence, the demarcation of abnormal from normal constituents of the body is another aim of the study of clinical biochemistry.

Clinical Biochemistry Laboratory: In the era of modern technology, health care delivery system involves so many different personnel and specialties that the caregiver must have an understanding and working knowledge of other professional endeavors, including the role of diagnostic evaluation. Basically, laboratory and diagnostic tests are tools by and of themselves, they are not therapeutic. In conjunction with a pertinent history and physical examination, these tests can confirm a diagnosis or provide valuable information about a patient status and response to therapy. In addition to these, laboratory findings are essential for epidemiological surveillance and research purposes.

Scopes Of Clinical Biochemistry: Biochemistry deals with the chemical nature, structure and functions of the chemicals of life so is involved in various research related works. It is used in clinical diagnosis, manufacture of various biological products, treatment of diseases ,in nutrition, agriculture. Also, biochemistry has revealed the abnormalities in their metabolism and their relationship to various diseases. Biochemistry has helped to correct these disorders with dietary modifications or genetic manipulations. Biochemistry has ramified into many branches of science like Medicine, pharmacy, Microbiology, Agriculture etc.

Role of medical/Clinical laboratory science in Health Care: The medical laboratory services play a pivotal role in the promotion, curative and preventive aspects of a nation's health delivery system. The service gives a scientific foundation by providing accurate information to those with the responsibility for: Treating patients and monitoring their response to treatment, Monitoring the development

and spread of infectious and dangerous pathogens (disease causing organisms), Deciding effective control measures against major prevalent disease,Without reliable laboratory services: The source of a disease may not be identified correctly. Patients are less likely to receive the best possible care. Resistance to essential drugs may develop and continue to spread. Epidemic diseases may not be identified on time and with confidence.

CODE OF CONDUCT FOR CLINICAL BIOCHEMISTRY LABORATORY PERSONNEL: Place the well-being and service of the sick above your own interests. Be loyal to your medical laboratory profession by maintain high standards of work and strive to improve your professional knowledge. Work scientifically and with complete honesty. Do not misuse your professional skills or knowledge for personal gain. Never take anything from your place of work that does not belong to you. Do not disclose to a patient or any unauthorized person the result of your investigations. Treat with utmost confidentiality and personal information that you may learn about a patient. Respect and work in harmony with the other members of your hospital staff or health center team.Be at all times courteous, patient, and considerate to the sick (patients) and their relations. Promote health care and the prevention and control of disease. Follow safety procedures and know how to apply first aid. Do not drink alcohol during laboratory working hours or when on emergency standby. Use equipment and laboratory-ware correctly and with care. Do not waste reagents or other laboratory supplies. Fulfil reliably and completely the terms and conditions of your employment.

Introduction to instrumentation and automation in clinical Biochemistry laboratories

“ALWAYS REMEMBER THAT YOU CAN BE A PATIENT TOMORROW, TREAT OTHERS AS YOU UNIVERSAL PRECAUTIONS TO BE TAKEN IN BIOCHEMISTRY LABORATORY

Following precautions should be applied on biochemistry lab: A. Safe laboratory premise Laboratory premise should be structurally sound with a reliable water supply and drainage from sink must be close to septic tank. The overall size of the laboratory must be appropriate for the work load, staff number, storage and equipment requirements.

The floor should be well constructed with a surface i.e. non slippery, impermeable to liquids and resistant to those chemicals used in the laboratory. Bench surface that are without crack, impervious, washable and resistant to the disinfections and chemical should be used in the laboratory. Suitable storage facility should be available including a ventilated lock store for the storage of chemical and expensive equipments.

The gas supply that that is piped into the laboratory with the gas cylinder should be stored in an outside weather proof. The hand basin with running water should be placed on the preferable site. Provision of protective safety cabinets and fume cupboards as required and when feasible. Safe electricity supple with sufficient wall electric points should be available.

- Along with fire extinguishers, several buckets of sand and a fire blanket is also required.
- B. Personal Health and safety measures

- Hands should be washed before and after handling the sample. Walking barefoot on the laboratory should be strictly prohibited. Eating, chewing gums, drinking, smoking, and applying cosmetics is also strictly prohibited. Avoid wearing jewellery in the working area, particularly pendant necklaces and bracelets.
- Mouth pipetting should be strictly prohibited. Personal equipments should not be kept on the working bench. Working area should be disinfected before and after performing the work.
- Basic Equipments Used In Biochemistry Laboratory: 1. Centrifuges 2. Water Baths 3. Hot Air Oven 4. Colorimeter 5. Spectrophotometer 6. Flame photometer 7. Micro Pipettes 8. Auto analyzer 9. Refrigerator 10.Computer etc.
- Centrifuge It is the separation technique used in clinical and research laboratories It is based on the principle of centrifugal force.
- The factors which govern the speed of centrifugation are:- The revolution per minute. Length of radius Shape and size of the particles. Viscosity and specific gravity of the fluid under centrifugation Gravitational force acting on the particles.
- Types I. Hand Centrifuge II. Motor Driven Centrifuge III. Micro - Hematocrit Centrifuge Hand Centrifuge Motor Driven Centrifuge Micro-Hematocrit Centrifuge
- Water Bath It is electrically heated and has a thermostatic temperature regulator Temperature ranging from room temperature to 100 ° C. Principle:- Water bath are made up of steel and are generally covered with electrostatic paint with high adherence and resistance to environmental laboratory conditions. It have an external panel on which a control can be found. They also have a tank made up of rustproof material with a collection of electrical resistors mounted on their lower parts. By the means of these, heat is transferred to the medium until reaching the temperature selected with a controlled device.
- Hot air oven It is a widely used method of sterilization by dry heat. Holding period is 160 ° C for 1 hour Is used to sterilize forceps, glassware, all glass syringes etc.
- Colorimeter It is an instrument used for the measurement of colored substance in solution. It involves the quantitative estimation of colour.
- Micro Pipettes Pipette is a laboratory tool commonly used in chemistry, biology and medicine to transport a measured volume of liquid often as a media dispenser. It consists a narrow tube into which fluid is drawn.
- Refrigerators:- Refrigerator is one of the most important equipment in laboratories. They maintain a temperature controlled environment for various fluid and substances. In laboratory, different kinds of refrigerators are used. They can be grouped by temperature ranges:- Conservation refrigerator in the range 2 to 8. Low temperature freezer in the range of -15 to -35. Ultra low temperature freezer in the range of -60 to -86. Principle:- Refrigerators function according to the law of physics regulating the energy transfer where temperature differences exist. From the second law of thermodynamics, it is known that if thermal energy needs to be transferred from a point with low temperature to another with high temperature, a mechanical task needs to be carried out. Modern refrigerators are thermal

system which function mainly using a cycle called compression, where refrigerators gas with special properties achieving heat transference is used.

. Semi auto- analyzer:- In case of these analyzer, the initial part of procedure like pipetting of reagent and specimen, mixing and incubation is carried out by the technician. Rest of procedure is carried out by the analyzer. Advantages:- Semi auto analyzer are cheap and compact compared to other fully automated analyzer. It need only 200 – 500µl of reagent per test. It can display and prints the result.

. Computer:- A computer is an electronic device that stores, retrieves, processes data, and can be programmed with instructions. A computer is composed of hardware & software, exist in a variety of sizes and configurations. Uses in laboratory:- They use computer to manage the request and specimen collection, control instrument, collect data, translate the data into meaningful results and generate report for physician information and inclusion in the patient's medical report.

. Glucose Estimations Renal Function Test • Urea • Creatinine • Sodium • Potassium • Uric acid Liver function test • Bilirubin • Protein • Albumin • Glutamate Pyruvate Transaminase (GPT) • Glutamate oxaloacetate transferase (GOT) • Alkaline Phosphate (ALP) Lipid Profile • Total Cholesterol • Triacylglycerol • High Density Lipo Protein (HDL) cholesterol • Low Density Lipo Protein (LDL) cholesterol Thyroid Function Test • Triiodothyronine (T3) • Thyronine (T4) • Thyroid Stimulating Hormones Cardiac Panel Test • Troponin • Creatinine Kinane (CKMB Test) • Lactate Dehydrogenase (LDH) • Myoglobin etc. . Amylase Estimation Calcium Estimation Phosphorus Estimation Biochemical test

HOT LABORATORIES
Electrolytes Bilirubin,Urea Aspartate transaminase,Glucose Alkaline phosphatase,Calcium, Turbidities,Creatinine Uric acid,Phosphate, Iron and IBC
COLD LABORATORY Electrolytes, Pigments,, Glucose Gastric analysis, Urea Toxicological tests, Amylase, Urine testing Neonatal Bilirubin pH/Po./Pco.
HORMONE LABORATORY
Urine 17-oxosteroids,Urine 17-hydroxycorticoids,Urine estrogens,Plasma cortisol,Protein Bound Iodine,Catecholamines, H.M.M.A.5 H.I.A.A., 5 Hydroxy-tryptamine,Magnesium
PROTEIN/LIPID LABORATORY Total proteins Cholesterol, Albumin Triglycerides Electrophoresis, Faecal fats, Chromoscanning Vitamin A absorption Immuno-electrophoresis tests Chromatography Iso-enzyrnes
ENZYME LABORATORY Transaminases Hydroxybutyrate dehydrogenase, Lactate dehydrogenase Isocitrate dehydrogenase Creatine phosphokinase Nucleotidase Acid phosphatase Pseudo-cholinesterase Trypsin and duodenal enzymes Pyruvate tolerance tests
RADIO-ISOTOPE LABORATORY:Thyroid uptake and excretion,Protein bound radio-iodine,Haematological tests and Blood volume

RESEARCH LABORATORY :Iso-enzymes Depends on research interests

Safety regulations and first aid.

Safety is an important aspect of any clinical laboratory. Every clinical laboratory must have a formal safety program to ensure a safe and healthy working environment. Safety should be the code of practice and it involves discipline in addressing the safe handling and containment of various hazardous materials in a laboratory . It is a joint responsibility of both management and laboratory workers and the duty of the management is to establish and maintain adequate standards, policies, procedures, work practices and maintenance of buildings and equipment. On the other hand, it is the responsibility of the laboratory workers to understand and adopt good laboratory practices to achieve a healthy working environment. A safe lab, in addition to protecting laboratory workers, also helps to protect the environment and public from exposure to hazardous materials(3). This addresses the various hazards encountered in a laboratory, its management, and the importance of safety program in a clinical laboratory set up is the first step towards safety regulations.The management should be committed to safety of its employees and it should increase health and safety awareness among employees through proper educational programs.

HAZARDS Hazards in a clinical laboratory can be broadly classified into chemical hazards, electrical hazards, fire hazards & biohazards.

CHEMICAL HAZARDS AND ITS MANAGEMENT

Common chemical hazards found in a lab are Explosives, Compressed gases, Flammables, Oxidizers, Toxic materials, and Corrosive materials.

FLAMMABLES: Substances which have a flash point or ignition point below room temperature. E.g. Oil and Gasoline, Ether etc. Storage rooms, cabinets and containers should be specially designed for such flammable liquids.

COMBUSTIBLES: Flash point at or above room temperature. It is better to choose a combustible product over a flammable product if all other considerations are equal. Clearing agents offer this choice.

EXPLOSIVES: Picric acid forms dangerous salts with certain metals which explode when wet (5). Avoid them altogether. Certain silver solutions, on ageing, explode by shaking. So never store these solutions after use.

OXIDATIVES: Oxidatives promote combustion in other materials, but are harmless themselves. They have a risk of fire hazard when in contact with suitable material. E.g. Sodium iodate , Mercuric oxides , Organic peroxides.

TOXIC MATERIALS: Causes death by ingestion, skin contact or inhalation, at certain specific concentration. E.g. Methanol- toxic, Formalin- toxic by ingestion and inhalation, Chromic acid, Osmium tetroxide and Uranyl nitratehighly toxic.

COMPRESSED GAS: Gas at room temperature (20°C) and pressure, packaged as a pressurized gas by compression or refrigeration and is usually quite heavy. The potential hazard of compressed gases occurs when sudden rupturing of the container causes it to become a dangerous projectile. E.g. Propane & Acetylene bottles.

CORROSIVE MATERIALS: Causes destruction of living tissue or irreversible alteration and destroy materials e.g. Bleach, Battery Acid, Ammonia & Hydrochloric Acid.

IRRITANTS: Reversible inflammatory effects at the site of contact. Eyes, skin and respiratory passages are affected. Formalin is a skin and respiratory irritant.

SENSITIZER: Causes allergic reaction. Sensitization lasts for life & gets worse with subsequent exposure. Formalin is a prime example.

CARCINOGENS: Chloroform, chromic acid, Dioxane, Formaldehyde, Nickel chloride, potassium dichromate, certain dyes etc.

CHEMICALS CAUSING TARGET

ORGAN EFFECTS: Cause specific harm selected anatomical or physiological systems.Xylene and toluene are neurotoxins. Benzene affects blood.Chloroform, Methanol, Xylene, Toluene are reproductive toxins.

MANAGEMENT OF CHEMICAL HAZARDS

Use cart to transport heavy or multiple number of containers from one area to another. A bottle should never be held by its neck, but instead firmly around its body, with one or both hands, depending on the size of the bottle to avoid spills. Acids must be diluted by slowly adding them to water while mixing; water should never be added to concentrated acid to avoid splattering. Acids, caustic materials and strong oxidizing agents should be mixed in the sink. This provides water for cooling as well as for confinement of the reagent in the event the flask or bottle breaks. Label the container before adding the reagent, and dispose off when proper expiry date is reached.No eating, drinking or smoking in the lab. Application of cosmetics is prohibited. Wash hands frequently but hydrate with a good lotion. Keep finger nails short. At the end of the day clean all working benches with a disinfectant. Wear closed-toed shoes.Tie back long hair. Do not wear sandals, jewelry, loose or baggy clothing

FIRST AID

Injuries caused by broken glass: Wash the wound immediately to remove any glass pieces. Apply mercurochrome or acriflavine ointment to the wound. Cover with gauze and adhesive tape.

Acid/Alkali splashes on the skin: Wash thoroughly; bath the affected skin with cotton wool soaked in 5% aqueous sodium carbonate if acid and 5% acetic acid or undiluted vinegar, if alkali.

Acid/Alkali splashes in the eye: Water spray from a wash bottle or rubber bulb into the medial corner of the eye . Put 4 drops of 2% Aqueous Sodium bicarbonate into the eye, if acid, and saturated solution of boric acid, if alkali.

Swallowing acid: Make the patient drink some 5% soap solution immediately. Make him gargle with the soap solution. Give him 3 or 4 glasses of ordinary water. If the lips and tongue are burned by the acid, rinse thoroughly with water. Bathe with 2% aqueous sodium bicarbonate.

Swallowing alkalies: Make the patient drink 5% solution of acetic acid or lemon juice or dilute vinegar. Make him gargle with the same acid solution. Give him 3 or 4 glasses of ordinary water. If the lips and tongue are burned by the alkali, rinse thoroughly with water; bathe with 5% acetic acid.

Poisoning :Send for a physician or qualified nurse,specifying the toxic substance involved. Place the victim in the open air while waiting for the physician.

Burns caused by heat: They fall into two categories

Severe burns: If the victim is on fire, roll him in a blanket or overall to smoothen the flames. Inform the physician. Lay the victim on the ground. Do not remove his clothing. Cover him if he is cold. Do not apply any treatment to the burns. This must be left to the physician.

Minor burns: Plunge the affected part into cold water or ice-water to soothe the pain. Apply Mercurochrome or Acriflavine ointment to the burn. Apply dry gauze dressing loosely. If the burn becomes infected or does not heal, refer the patient to a physician. Never tear off the blisters that form over the burns.

ELECTRICAL HAZARD AND ITS MANAGEMENT

Electrical equipment should not be handled with wet hands, nor should electrical equipment be used after liquid has been spilled on it. The equipment must be turned off immediately and dried thoroughly. In case of a wet or malfunctioning electrical

instrument the plug should be pulled and a note of cautioning should be left on the instrument. Use of extension cords is
Prohibited.

Bodily damage by electric shock: The symptoms are fainting and asphyxia. Before doing anything else, put off the main switch. Send for a physician. Begin giving mouth to mouth respiration immediately.

FIRE HAZARD AND ITS MANAGEMENT :Fire in the laboratory may occur due to spirit lamps, electrical appliances or other inflammable reagents used in a laboratory. All laboratories should have a fire extinguisher and easy access to safety showers and fire blankets. For putting off the flames from the inflammable liquids, smoothen the fire by throwing sand over it.

BIOHAZARDS

Can be infectious agents themselves or items (solutions, specimens or objects) contaminated with anything that can cause disease in humans regardless of its source. To operate a clinical laboratory safely, it is essential to prevent the exposure of laboratory workers to infectious agents such as the hepatitis B virus (HBV) and the human immunodeficiency virus (HIV).

Universal Precautions : specify how clinical laboratories handle infectious
agents. In general, they mandate that clinical laboratories treat all human blood and other potentially infectious materials as if they were known to contain infectious pathogens. The specifications apply to all specimens of blood, serum, plasma, blood products, vaginal secretions, semen, cerebrospinal fluid, synovial fluid and concentrated HBV or HIV viruses. In addition, any specimen that contains visible traces of blood should be handled using these Universal Precautions. Universal Precautions also specifies that barrier protection must be used by laboratory workers to prevent skin and mucous membrane contamination from specimens. These barriers, also known as personal protective equipment (PPE) include gloves, gowns, laboratory coats, face shields or mask and eye protection, mouth pieces, resuscitation bags, pocket masks, or other ventilator devices.

PRECAUTIONS FOR BIOLOGICAL HAZARDS

Never perform mouth pipetting and never blow out pipettes that contain
potentially infectious material. Barrier protections such as gloves, masks, and protective eye wear and gowns are to be worn. Wash hands whenever gloves are changed .Facial barrier protection should be used if there is a significant potential for the spattering of blood or body fluids.

Dispose off needles in rigid containers; use the "one-handed" technique. Dispose of all sharps appropriately. Encourage frequent hand washing in the laboratory; employees must wash their hands whenever they leave the laboratory. Make a habit of keeping hands away from your mouth, nose, eyes, and any other mucous membranes. This reduces the possibility of self inoculation. Decontaminate all surfaces and reusable devices after use with appropriate hospital disinfectants. Use proper biohazard disposal techniques (e.g., Red Bag) . Never leave a discarded tube or infected material unattended or unlabeled. Periodically clean out freezer and dry-ice chests to remove broken ampules and tubes of biological specimens.Hepatitis B vaccine be offered to all employees at risk of potential exposure as a regular or occasional part of their duties.

SPECIAL NOTE FOR HISTOLOGY LABS

Fresh specimens of human origin must always be considered potentially infectious. Grossing an unfixed specimen is the most risk activity that can be undertaken in a histology lab .Fixed specimens have a much reduced risk; nearly all infectious agents

are readily deactivated by fixation provided the specimen is thoroughly fixed for proper time. Tissue in the first several stations of a tissue processor may remain bio hazardous. Complete penetration by alcohol will kill all infectious agents except prions. So properly processed specimens can be handled without special precautions. Cryotomy carries special risk because tissue is always fresh & small dust-like particles generated from sectioning may become air borne

CONTAMINATION BY INFECTED MATERIAL

In case of wounds caused by broken glassware containing stools, pus, etc., wash the wound immediately with antiseptic lotion. Check whether the cut is bleeding. If not, squeeze hard to make it bleed for several minutes. Refer the patient to a physician if the material involved is

known to be very infective, e.g. pus. If infected material is accidentally sucked into the mouth spit it out immediately. Use a disinfectant (e.g. Diluted Dettol) for mouth washing (8) . If the infected material has been swallowed accidentally, forced vomiting is to be done. Ascertain the kind of infection and take advice from a medical person.

The responsibilities of management are to anticipate problems and to develop safety procedures and training programs based on present or potential hazards that may endanger personnel and on the behavioural factors leading to unsafe acts. Safety awareness should become habit and a way.

Blood Specimen Collection and Processing

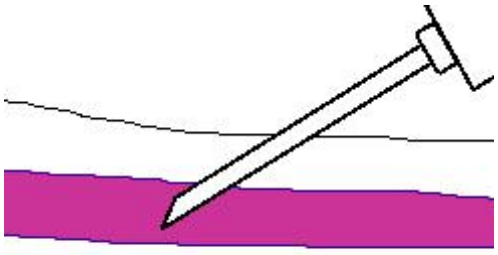
The first step in acquiring a quality lab test result for any patient is the specimen collection procedure. The venipuncture procedure is complex, requiring both knowledge and skill to perform. Several essential steps are required for every successful collection procedure:

Venipuncture Procedure:

1. A phlebotomist must have a professional, courteous, and understanding manner in all contact with all patients.
2. The first step to the collection is to positively identify the patient by two forms of identification; ask the patient to state and spell his/her name and give you his/her birth date. Check these against the requisition (paper or electronic).
3. Check the requisition form for requested tests, other patient information and any special draw requirements. Gather the tubes and supplies that you will need for the draw.
4. Position the patient in a chair, or sitting or lying on a bed.
5. Wash your hands.
6. Select a suitable site for venipuncture, by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient.
7. Do not put the tourniquet on too tightly or leave it on the patient longer than 1 minute.
8. Next, put on non-latex gloves, and palpate for a vein.
9. When a vein is selected, cleanse the area in a circular motion, beginning at the site and working outward. Allow the area to air dry. After the area is cleansed, it should not be touched or palpated again. If you find it necessary to

reevaluate the site by palpation, the area needs to be re-cleansed before the venipuncture is performed.

10. Ask the patient to make a fist; avoid "pumping the fist." Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. Swiftly insert the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface. Avoid excess probing.



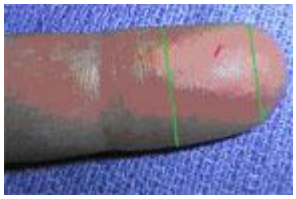
11. When the last tube is filling, remove the tourniquet.
12. Remove the needle from the patient's arm using a swift backward motion.
13. Place gauze immediately on the puncture site. Apply and hold adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, tape a fresh piece of gauze or Band-Aid to the puncture site.
14. Dispose of contaminated materials/supplies in designated containers.

Note: The larger median cubital and cephalic veins are the usual choice, but the basilic vein on the dorsum of the arm or dorsal hand veins are also acceptable. Foot veins are a last resort because of the higher probability of complications.

Fingerstick Procedure:

1. Follow steps #1 through #5 of the procedure for venipuncture as outlined above.
2. The best locations for fingersticks are the 3rd (middle) and 4th (ring) fingers of the non-dominant hand. Do not use the tip of the finger or the center of the finger. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash.
3. When a site is selected, put on gloves, and cleanse the selected puncture area.
4. Massage the finger toward the selected site prior to the puncture.
5. Using a sterile safety lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.

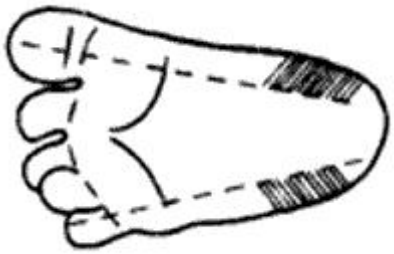
6. Wipe away the first drop of blood, which tends to contain excess tissue fluid.



7. Collect drops of blood into the collection tube/device by gentle pressure on the finger. Avoid excessive pressure or “milking” that may squeeze tissue fluid into the drop of blood.
8. Cap, rotate and invert the collection device to mix the blood collected.
9. Have the patient hold a small gauze pad over the puncture site for a few minutes to stop the bleeding.
10. Dispose of contaminated materials/supplies in designated containers.
11. Label all appropriate tubes at the patient bedside.

Heelstick Procedure (infants):

The recommended location for blood collection on a newborn baby or infant is the heel. The diagram below indicates the proper area to use for heel punctures for blood collection.



1. Prewarming the infant's heel (42° C for 3 to 5 minutes) is important to increase the flow of blood for collection.
2. Wash your hands, and put gloves on. Clean the site to be punctured with an alcohol sponge. Dry the cleaned area with a dry gauze pad.
3. Hold the baby's foot firmly to avoid sudden movement.
4. Using a sterile blood safety lancet, puncture the side of the heel in the appropriate regions shown above. Make the cut across the heel print lines so that a drop of blood can well up and not run down along the lines.
5. Wipe away the first drop of blood with a piece of clean, dry cotton gauze. Since newborns do not often bleed immediately, use gentle pressure to produce a rounded drop of blood. Do not use excessive pressure because the blood may become diluted with tissue fluid.
6. Fill the required microtainer(s) as needed.

7. When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped. Apply tape or Band-Aid to area if needed.
8. Be sure to dispose of the lancet in the appropriate sharps container. Dispose of contaminated materials in appropriate waste receptacles.
9. Remove your gloves and wash your hands.

Order of Draw:

Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes. The recommended order of draw is:

1. First - blood culture bottle or tube (yellow or yellow-black top)
2. Second - coagulation tube (light blue top).
3. Third - non-additive tube (red top)
4. Last draw - additive tubes in this order:

    1. SST (red-gray or gold top). Contains a gel separator and clot activator.
    2. Sodium heparin (dark green top)
    3. PST (dark green green top with gold rim). Contains lithium heparin anticoagulant and a gel separator.
    4. EDTA (lavender top)
    5. Oxalate/fluoride (light gray top) or other additives

    NOTE: Tubes with additives must be thoroughly mixed. Clotting or erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.

Labeling The Sample

All specimens must be received by the laboratory with a legible label containing at least two (2) unique identifiers.

The specimen must be labeled with the patient's full name (preferably last name first, then first name last) and one of the following:

- Geisinger medical record number (MRN) - for Geisinger locations, this is the required second identifier
- Patient's full date of birth (must include the month, day, and year)
- Unique requisition identifier/label

Areas to Avoid When Choosing a Site for Blood Draw:

Certain areas are to be avoided when choosing a site for blood draw:

- Extensive scars from burns and surgery - it is difficult to puncture the scar tissue and obtain a specimen.

- The upper extremity on the side of a previous mastectomy - test results may be affected because of lymphedema.
- Hematoma - may cause erroneous test results. If another site is not available, collect the specimen distal to the hematoma.
- Intravenous therapy (IV) / blood transfusions - fluid may dilute the specimen, so collect from the opposite arm if possible.
- Cannula/fistula/heparin lock - hospitals have special policies regarding these devices. In general, blood should not be drawn from an arm with a fistula or cannula without consulting the attending physician.
- Edematous extremities - tissue fluid accumulation alters test results.

Techniques to Prevent Hemolysis (which can interfere with many tests):

- Mix all tubes with anticoagulant additives gently (vigorous shaking can cause hemolysis) 5-10 times.
- Avoid drawing blood from a hematoma; select another draw site.
- If using a needle and syringe, avoid drawing the plunger back too forcefully.
- Make sure the venipuncture site is dry before proceeding with draw.
- Avoid a probing, traumatic venipuncture.
- Avoid prolonged tourniquet application (no more than 2 minutes; less than 1 minute is optimal).
- Avoid massaging, squeezing, or probing a site.
- Avoid excessive fist clenching.
- If blood flow into tube slows, adjust needle position to remain in the center of the lumen.

Blood Sample Handling and Processing:

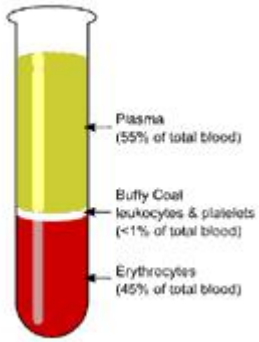
Pre-centrifugation Handling - The first critical step in the lab testing process, after obtaining the sample, is the preparation of the blood samples. Specimen integrity can be maintained by following some basic handling processes:

- Fill tubes to the stated draw volume to ensure the proper blood-to-additive ratio. Allow the tubes to fill until the vacuum is exhausted and blood flow ceases.
- Tubes should be stored at 4-25°C (39-77°F).
- Tubes should not be used beyond the designated expiration date.
- Mix all gel barrier and additive tubes by gentle inversion 5 to10 times immediately after the draw. This assists in the clotting process. This also assures homogenous mixing of the additives with the blood in all types of additive tubes.
- Serum separator tubes should clot for a full 30 minutes in a vertical position prior to centrifugation. Short clotting times can result in fibrin formation, which may interfere with complete gel barrier formation.

Blood Sample Centrifugation – It is recommended that serum be physically

separated from contact with cells as soon as possible, with a maximum time limit of 2 hours from the time of collection.

- Complete gel barrier formation (gel barrier tubes) is time, temperature and G-force dependent. The uniformity of the barrier is time dependent; an incomplete barrier could result from shortened centrifugation times.



- In general, for a horizontal, swing-bucket centrifuge, the recommended spin time is 10 minutes. For a fixed-angle centrifuge, the recommended spin time is 15 minutes.
- NOTE: Gel flow may be impeded if chilled before or after centrifugation.
- Tubes should remain closed at all times during the centrifugation process.
- Place the closed tubes in the centrifuge as a “balanced load” noting the following:

    - Opposing tube holders must be identical and contain the same cushion or none at all.
    - Opposing tube holders must be empty or loaded with equally weighted samples (tubes of the same size and equal in fill).
    - If an odd number of samples is to be spun, fill a tube with water to match the weight of the unpaired sample and place it across from this sample.

Centrifuge Safety

- Interference with an activated centrifuge by an impatient employee can result in bodily injury in the form of direct trauma or aerosolization of hazardous droplets.
- Centrifuges must never be operated without a cover in place.
- Uncovered specimen tubes must not be centrifuged.
- Centrifuges must never be slowed down or stopped by grasping part(s) of the device with your hand or by applying another object against the rotating equipment.
- Be sure the centrifuge is appropriately balanced before activating. If an abnormal noise, vibration, or sound is noted while the centrifuge is in

operation, immediately stop the unit (turn off the switch) and check for a possible load imbalance.

- Clean the centrifuge daily with a disinfectant and paper towel. Broken tubes or liquid spills must be cleaned immediately.

Transport of specimen

1.Specimen Transport: To minimize exposure to bloodborne pathogens in transport of specimens, Standard Precautions must be used. ALL blood and other potentially infectious material are treated as if they are known to be infectious with HIV or hepatitis and other bloodborne pathogens. All specimens must be transported in a sealed biohazard bag for specific storage requirements room temp, (ambient) : refrigeration, or frozen) for the testing of the patient sample.
2.Specimen Collection, Handling and Transport Room Temp Requirements: If your specimen does not have a specific storage requirement and will be stored at room temp before Courier pickup, please place in a sealed orange/red biohazard labeled specimen bag.
Note: Do not store tubes in direct contact of a heat source such as direct sunlight, top of refrigerator, heating/air vents, etc.
3.Refrigeration: If your specimen requires refrigerated temperatures during transport, place in a “blue” biohazard labeled specimen bag. Please use a permanent marker and place an “X” in the box designating “Refrigerator” temperature. Place the specimen in your refrigerator until Courier pickup. The blue bag will alert the Courier that this specimen will need to be transported on ice to retain the refrigerator temperature.
Note: Never store unspun serum/plasma tubes in refrigerator. Tubes must be centrifuged before storage to ensure specimen integrity.
4.Frozen: If your test requires the specimen to be frozen after processing, the specimen must be centrifuged and serum/plasma must be transferred to an aliquot tube by pipette without disturbing gel or packed cells. Following labeling requirements for all aliquots. The aliquot is to be placed in a blue biohazard labeled specimen bag. Please use a permanent marker and place an “X” in the box designating “Frozen” temperature. Place specimen bag in your freezer (or on dry ice) until Courier pickup. The blue bag will alert the Courier that this specimen will need to be transported on dry ice to retain the frozen temperature. Note: Never place original collected tube in freezer. Freeze only the labeled aliquot sample.
5.STAT: If your specimen has a “STAT” priority, please call your Courier for pickup. Place the sample in a “Red” biohazard labeled specimen bag. The red bag will easily be seen as a STAT specimen upon delivery to the laboratory.
6.Other Requirements:
Remove all needles and sharps from all specimens before transporting. Specimen Collection, Handling and Transport All specimens transported via courier must be transported in sealed biohazard, leak-proof, puncture resistant container tightly closed before transportation.
Please place specimens in the Ziploc portion of the specimen bag. Completed requisition is to be placed in the outside pocket

Concepts of accuracy, precision, test sensitivity, test specificity in the quantitative assessment of test performance

Diagnosis tests include different kinds of information, such as medical tests (e.g. blood tests, X-rays, MRA), medical signs (clubbing of the fingers, a sign of lung disease), or symptoms (e.g. pain in a particular pattern). Doctor's decisions of medical treatment rely on diagnosis tests, which makes the accuracy of a diagnosis is essential in medical care. Fortunately, the attributes of the diagnosis tests can be measured. For a given disease condition, the best possible test can be chosen based on these attributes. Sensitivity, specificity and accuracy are widely used statistics to describe a diagnostic test. In particular, they are used to quantify how good and reliable a test is. Sensitivity evaluates how good the test is at detecting a positive disease. Specificity estimates how likely patients without disease can be correctly ruled out. ROC curve is a graphic presentation of the relationship between both sensitivity and specificity and it helps to decide the optimal model through determining the best threshold for the diagnostic test.

Accuracy measures how correct a diagnostic test identifies and excludes a given condition. Accuracy of a diagnostic test can be determined from sensitivity and specificity with the presence of prevalence. Given the importance of these statistics in disease diagnosis and the terms are easily confused, it is important to get familiar with how they work, it helps us better understand when to use, how to implement them, and how to interpret the results. The importance and popularity of these statistics urges for a thorough review along with practical SAS examples.

This will focus on the concepts of sensitivity, specificity and accuracy in the context of disease diagnosis: starting with a review of the definitions , how to calculate sensitivity, specificity and accuracy, associated 95% confidence interval and ROC analysis; followed by a practical example of disease diagnosis and related SAS macro code; then moving on to the common issues on interpreting the results of sensitivity, specificity and accuracy; ended by a final remark of the entire paper. 2. SENSITIVITY, SPECIFICITY AND ACCURACY,

Condition (e.g. Disease) As determined by the Standard of Truth Outcome of the diagnostic test Positive Negative Row Total Positive TP FP TP+FP (Total number of subjects with positive test) Negative FN TN FN + TN (Total number of subjects with negative test) Column total TP+FN (Total number of subjects with given condition) FP+TN (Total number of subjects without given condition) N = TP+TN+FP+FN (Total number of subjects in study) Table 1. Terms used to define sensitivity, specificity and accuracy 2 There are several terms that are commonly used along with the description of sensitivity, specificity and accuracy. They are true positive (TP), true negative (TN), false negative (FN), and false positive (FP). If a disease is proven present in a patient, the given diagnostic test also indicates the presence of disease, the result of the diagnostic test is considered true positive. Similarly, if a disease is proven absent in a patient, the diagnostic test suggests the disease is absent as well, the test result is true negative (TN). Both true positive and true negative suggest a consistent result between the diagnostic test and the proven condition (also called standard of truth). However, no medical test is perfect. If the diagnostic test indicates the presence of disease in a patient who actually has no such disease, the test result is false positive (FP). Similarly, if the result of the diagnosis test suggests that the disease is absent for a patient with disease for sure, the test result is false negative (FN). Both false positive and false negative indicate that the test results are opposite to the actual

condition. Sensitivity, specificity and accuracy are described in terms of TP, TN, FN and FP.
Sensitivity = TP/(TP + FN) = (Number of true positive assessment)/(Number of all positive assessment)

Specificity = TN/(TN + FP) = (Number of true negative assessment)/(Number of all negative assessment) Accuracy = (TN + TP)/(TN+TP+FN+FP) = (Number of correct assessments)/Number of all assessments)
As suggested by above equations, sensitivity is the proportion of true positives that are correctly identified by a diagnostic test. It shows how good the test is at detecting a disease. Specificity is the proportion of the true negatives correctly identified by a diagnostic test. It suggests how good the test is at identifying normal (negative) condition. Accuracy is the proportion of true results, either true positive or true negative, in a population. It measures the degree of veracity of a diagnostic test on a condition. The numerical values of sensitivity represents the probability of a diagnos tic test identifies patients who do in fact have the disease. The higher the numerical value of sensitivity, the less likely diagnos tic test returns false-positive results. For example, if sensitivity = 99%, it means: when we conduct a diagnostic test on a patient with certain disease, there is 99% of chance, this patient will be identified as positive. A test with high sensitivity tents to capture all possible positive conditions without missing anyone. Thus a test with high sensitivity is often used to screen for disease. The numerical value of specificity represents the probability of a test diagnoses a particular disease without giving false-positive results. For example, if the specificity of a test is 99%. It means: when we conduct a diagnostic test on a patient without certain disease, there is 99% chance; this patient will be identified as negative. A test can be very specific without being sensitive, or it can be very sensitive without being specific. Both factors are equally important. A good test is a one has both high sensitivity and specificity. A good example of a test with high sensitive and specificity is pregnancy test. A positive result of pregnancy test almost for sure suggests the subject who took the test is pregnant. A negative result almost certainly rules out the possibility of being pregnant. In addition to the equation show above, accuracy can be determined from sensitivity and specificity, where prevalence is known. Prevalence is the probability of disease in the population at a given time: Accuracy = (sensitivity) (prevalence) + (specificity) (1 - prevalence). The numerical value of accuracy represents the proportion of true positive results (both true positive and true negative) in the selected population. An accuracy of 99% of times the test result is accurate, regardless positive or negative. This stays correct for most of the cases. However, it worth mentioning, the equation of accuracy implies that even if both sensitivity and specificity are high, say 99%, it does not suggest that the accuracy of the test is equally high as well. In addition to sensitivity and specificity, the accuracy is also determined by how common the disease in the selected population. A diagnosis for rare conditions in the population of interest may result in high sensitivity and specificity, but low accuracy. Accuracy needs to be interpreted cautiously

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SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLGOY

UNIT – II - HEMATOLOGY - SBC3104

UNIT 2 HEMATOLOGY 9 Hrs

Blood:- composition and their functions, Anemia:- classifications, erythrocyte indices. Blood coagulation system, Clotting time, Bleeding time, RBC count, WBC count, cot, determination of Hb, PCV and ESR.Hemoglobinopathies, Thalassemias.

UNIT-II

HEMATOLOGY

Blood facts

Approximately 8% of an adult's body weight is made up of blood. Females have around 4-5 litres, while males have around 5-6 litres. This difference is mainly due to the differences in body size between men and women.Its mean temperature is 38 degrees Celcius.It has a pH of 7.35-7.45, making it slightly basic (less than 7 is considered acidic).Whole blood is about 4.5-5.5 times as viscous as water, indicating that it is more resistant to flow than water. This viscosity is vital to the function of blood because if blood flows too easily or with too much resistance, it can strain the heart and lead to severe cardiovascular problems.Blood in the arteries is a brighter red than blood in the veins because of the higher levels of oxygen found in the arteries.An artificial substitute for human blood has not been found.

Functions of blood

Blood has three main functions: transport, protection and regulation.

Transport

Blood transports the following substances:Gases, namely oxygen (O_2) and carbon dioxide (CO_2), between the lungs and rest of the body.Nutrients from the digestive tract and storage sites to the rest of the body.Waste products to be detoxified or removed by the liver and kidneys.Hormones from the glands in which they are produced to their target cells Heat to the skin so as to help regulate body temperature

Protection

Blood has several roles in inflammation:Leukocytes, or white blood cells, destroy invading microorganisms and cancer cells.Antibodies and other proteins destroy pathogenic substances.Platelet factors initiate blood clotting and help minimise blood loss

Regulation

Blood helps regulate:pH by interacting with acids and bases.Water balance by transferring water to and from tissues

Composition of blood

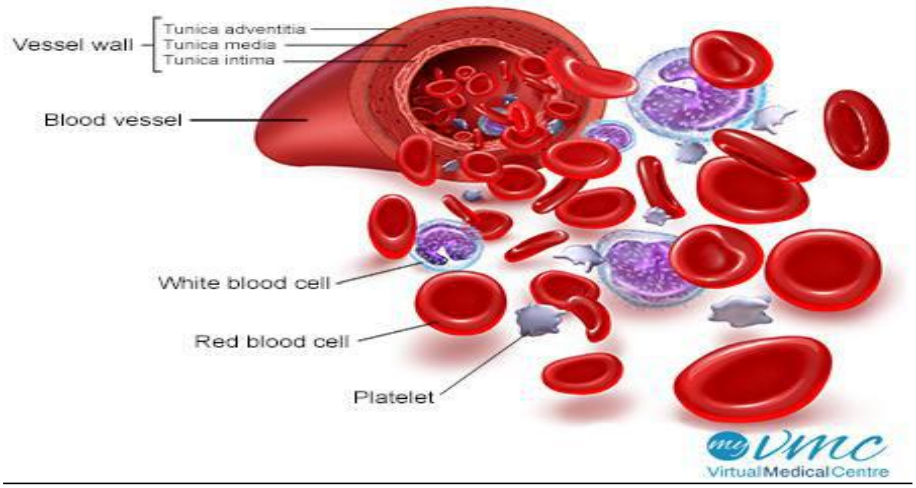
Blood is classified as a connective tissue and consists of two main components:Plasma, which is a clear extracellular fluid Formed elements, which are made up of the blood cells and platelets.The formed elements are so named because they are enclosed in a plasma membrane and have a definite structure and shape. All formed elements are cells except for the platelets, which are tiny fragments of bone marrow cells.

Formed elements are:

Erythrocytes, also known as red blood cells (RBCs)

Leukocytes, also known as white blood cells (WBCs)

Platelets



Leukocytes are further classified into two subcategories called granulocytes which consist of neutrophils, eosinophils and basophils; and agranulocytes which consist of lymphocytes and monocytes.

The formed elements can be separated from plasma by centrifuge, where a blood sample is spun for a few minutes in a tube to separate its components according to their densities. RBCs are denser than plasma, and so become packed into the bottom of the tube to make up 45% of total volume. This volume is known as the haematocrit. WBCs and platelets form a narrow cream-coloured coat known as the buffy coat immediately above the RBCs. Finally, the plasma makes up the top of the tube, which is a pale yellow colour and contains just under 55% of the total volume.

Blood plasma

Blood plasma is a mixture of proteins, enzymes, nutrients, wastes, hormones and gases. The specific composition and function of its components are as follows:
Proteins
These are the most abundant substance in plasma by weight and play a part in a variety of roles including clotting, defence and transport. Collectively, they serve several functions:

- They are an important reserve supply of amino acids for cell nutrition. Cells called macrophages in the liver, gut, spleen, lungs and lymphatic tissue can break down plasma proteins so as to release their amino acids. These amino acids are used by other cells to synthesise new products.
- Plasma proteins also serve as carriers for other molecules. Many types of small molecules bind to specific plasma proteins and are transported from the organs that absorb these proteins to other tissues for utilisation. The proteins also help to keep the blood slightly basic at a stable pH. They do this by functioning as weak bases themselves to bind excess H+ ions. By doing so, they remove excess H+ from the blood which keeps it slightly basic.
- The plasma proteins interact in specific ways to cause the blood to coagulate, which is part of the body's response to injury to the blood vessels (also known as

vascular injury), and helps protect against the loss of blood and invasion by foreign microorganisms and viruses.

- Plasma proteins govern the distribution of water between the blood and tissue fluid by producing what is known as a colloid osmotic pressure.

  There are three major categories of plasma proteins, and each individual type of proteins has its own specific properties and functions in addition to their overall collective role:

Albumins, which are the smallest and most abundant plasma proteins. Reductions in plasma albumin content can result in a loss of fluid from the blood and a gain of fluid in the interstitial space (space within the tissue), which may occur in nutritional, liver and kidney disease. Albumin also helps many substances dissolve in the plasma by binding to them, hence playing an important role in plasma transport of substances such as drugs, hormones and fatty acids.

Globulins, which can be subdivided into three classes from smallest to largest in molecular weight into alpha, beta and gamma globulins. The globulins include high density lipoproteins (HDL), an alpha-1 globulin, and low density lipoproteins (LDL), a beta-1 globulin. HDL functions in lipid transport carrying fats to cells for use in energy metabolism, membrane reconstruction and hormone function. HDLs also appear to prevent cholesterol from invading and settling in the walls of arteries. LDL carries cholesterol and fats to tissues for use in manufacturing steroid hormones and building cell membranes, but it also favours the deposition of cholesterol in arterial walls and thus appears to play a role in disease of the blood vessels and heart. HDL and LDL therefore play important parts in the regulation of cholesterol and hence have a large impact on cardiovascular disease.

Fibrinogen, which is a soluble precursor of a sticky protein called fibrin, which forms the framework of blood clot. Fibrin plays a key role in coagulation of blood, which is discussed later in this article under Platelets.

Amino acidsThese are formed from the break down of tissue proteins or from the digestion of digested proteins.

Nitrogenous wasteBeing toxic end products of the break down of substances in the body, these are usually cleared from the bloodstream and are excreted by the kidneys at a rate that balances their production.

NutrientsThose absorbed by the digestive tract are transported in the blood plasma. These include glucose, amino acids, fats, cholesterol, phospholipids, vitamins and minerals.

GasesSome oxygen and carbon dioxide are transported by plasma. Plasma also contains a substantial amount of dissolved nitrogen.

Electrolytes

The most abundant of these are sodium ions, which account for more of the blood's osmolarity than any other solute.Red blood cells (RBCs), also known as erythrocytes, have two main functions:

To pick up oxygen from the lungs and deliver it to tissues elsewhere,To pick up carbon dioxide from other tissues and unload it in the lungs.An erythrocyte is a disc-shaped cell with a thick rim and a thin sunken centre. The plasma membrane of a mature RBC has glycoproteins and glycolipids that determine a person's blood type. On its inner surface are two proteins called spectrin and actin that give the membrane resilience and durability. This allows the RBCs to stretch, bend and fold as they squeeze through small blood vessels, and to spring back to their original shape as they pass through larger vessels.RBCs are incapable of aerobic respiration, preventing them from consuming the

oxygen they transport because they lose nearly all their inner cellular components during maturation. The inner cellular components lost include their mitochondria, which normally provide energy to a cell, and their nucleus, which contains the genetic material of the cell and enable it to repair itself. The lack of a nucleus means that RBCs are unable to repair themselves. However, the resulting biconcave shape is that the cell has a greater ratio of surface area to volume, enabling O_2 and CO_2 to diffuse quickly to and from Hb.The cytoplasm of a RBC consists mainly of a 33% solution of haemoglobin (Hb), which gives RBCs their red colour. Haemoglobin carries most of the oxygen and some of the carbon dioxide transported by the blood.Circulating erythrocytes live for about 120 days. As a RBC ages, its membrane grows increasingly fragile. Without key organelles such as a nucleus or ribosomes, RBCs cannot repair themselves. Many RBCs die in the spleen, where they become trapped in narrow channels, broken up and destroyed. Haemolysis refers to the rupture of RBCs, where haemoglobin is released leaving empty plasma membranes which are easily digested by cells known as macrophages in the liver and spleen. The Hb is then further broken down into its different components and either recycled in the body for further use or disposed of.

White blood cells

White blood cells (WBCs) are also known as leukocytes. They can be divided into granulocytes and agranulocytes. The former have cytoplasms that contain organelles that appear as coloured granules through light microscopy, hence their name. Granulocytes consist of neutrophils, eosinophils and basophils. In contrast, agranulocytes do not contain granules. They consist of lymphocytes and monocytes.

Granulocytes

Neutrophils: These contain very fine cytoplasmic granules that can be seen under a light microscope. Neutrophils are also called polymorphonuclear (PMN) because they have a variety of nuclear shapes. They play roles in the destruction of bacteria and the release of chemicals that kill or inhibit the growth of bacteria.

Eosinophils: These have large granules and a prominent nucleus that is divided into two lobes. They function in the destruction of allergens and inflammatory chemicals, and release enzymes that disable parasites.

Basophils: They have a pale nucleus that is usually hidden by granules. They secrete histamine which increases tissue blood flow via dilating the blood vessels, and also secrete heparin which is an anticoagulant that promotes mobility of other WBCs by preventing clotting.

Agranulocytes

Lymphocytes: These are usually classified as small, medium or large. Medium and large lymphocytes are generally seen mainly in fibrous connective tissue and only occasionally in the circulation bloodstream. Lymphocytes function in destroying cancer cells, cells infected by viruses, and foreign invading cells. In addition, they present antigens to activate other cells of the immune system. They also coordinate the actions of other immune cells, secrete antibodies and serve in immune memory.

Monocytes: They are the largest of the formed elements. Their cytoplasm tends to be abundant and relatively clear. They function in differentiating into macrophages, which are large phagocytic cells, and digest pathogens, dead neutrophils, and the debris of dead cells. Like lymphocytes, they also present antigens to activate other immune cells.

Platelets

Platelets are small fragments of bone marrow cells and are therefore not really classified as cells themselves.Platelets have the following functions:Secrete vasoconstrictors which constrict blood vessels, causing vascular spasms in broken blood vessels

Form temporary platelet plugs to stop bleeding
Secrete procoagulants (clotting factors) to promote blood clotting
Dissolve blood clots when they are no longer needed
Digest and destroy bacteria
Secrete chemicals that attract neutrophils and monocytes to sites of inflammation
Secrete growth factors to maintain the linings of blood vesselsThe first three functions listed above refer to important haemostatic mechanisms in which platelets play a role in during bleeding: vascular spasms, platelet plug formation and blood clotting (coagulation).

Vascular spasm

This is a prompt constriction of the broken blood vessel and is the most immediate protection against blood loss. Injury stimulates pain receptors. Some of these receptors directly innervate nearby blood vessels and cause them to constrict. After a few minutes, other mechanisms take over. Injury to the smooth muscle of the blood vessel itself causes a longer-lasting vasoconstriction where platelets release a chemical vasoconstrictor called serotonin. This maintains vascular spasm long enough for the other haemostatic mechanisms to come into play.

Platelet plug formation

Under normal conditions, platelets do not usually adhere to the wall of undamaged blood vessels, since the vessel lining tends to be smooth and coated with a platelet repellent. When a vessel is broken, platelets put out long spiny extensions to adhere to the vessel wall as well as to other platelets. These extensions then contract and draw the walls of the vessel together. The mass of platelets formed is known as a platelet plug, and can reduce or stop minor bleeding.

Coagulation

This is the last and most effective defence against bleeding. During bleeding, it is important for the blood to clot quickly to minimise blood loss, but it is equally important for blood not to clot in undamaged vessels. Coagulation is a very complex process aimed at clotting the blood at appropriate amounts. The objective of coagulation is to convert plasma protein fibrinogen into fibrin, which is a sticky protein that adheres to the walls of a vessel. Blood cells and platelets become stuck to fibrin, and the resulting mass helps to seal the break in the blood vessel. The forming of fibrin is what makes coagulation so complicated, as it involved numerous chemicals reactions and many coagulation factors.

Production of blood

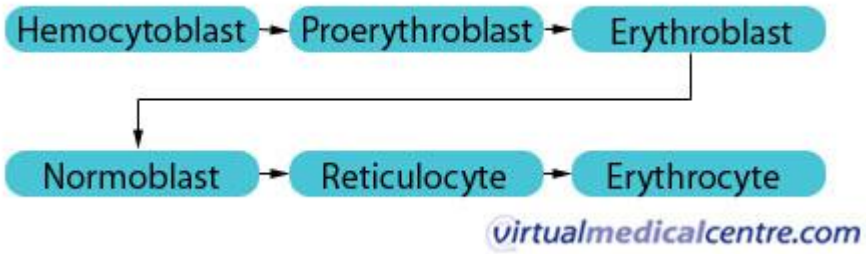
Haemopoiesis

Haemopoiesis is the production of the formed elements of blood. Haemopoietic tissues refer to the tissues that produce blood. The earliest haemopoietic tissue to develop is the yolk sac, which also functions in the transfer of yolk nutrients of the

embryo. In the foetus, blood cells are produced by the bone marrow, liver, spleen and thymus. This changes during and after birth. The liver stops producing blood cells around the time of birth, while the spleen stops producing them soon after birth but continues to produce lymphocytes for life. From infancy onwards, all formed elements are produced in the red bone marrow. Lymphocytes are additionally produced in lymphoid tissues and organs widely distributed in the body, including the thymus, tonsils, lymph nodes, spleen and patches of lymphoid tissues in the intestine.

Erythropoesis

Erythropoiesis refers specifically to the production of erythrocytes or red blood cells (RBCs). These are formed through the following sequence of cell transformations:



The proerythroblast has receptors for the hormone erythropoietin (EPO). Once EPO receptors are in place, the cell is committed to exclusively producing RBCs. The erythroblasts then multiply and synthesise haemoglobin (Hb), which is a red oxygen transport protein. The nucleus from the erythroblasts is then discarded, giving rise to cells named reticulocytes. The overall transformation from haemocytoblast to reticulocytes involves a reduction in cell size, an increase in cell number, the synthesis of haemoglobin, and the loss of the cell nucleus. These reticulocytes leave the bone marrow and enter the bloodstream where they mature into erythrocytes when their endoplasmic reticulum disappears.

Leukopoiesis

Leukopoiesis refers to the production of leukocytes (WBCs). It begins when some types of haemocytoblasts differentiate into three types of committed cells:

. B progenitors, which are destined to become B lymphocytes
. T progenitors, which become T lymphocytesGranulocyte-macrophage colony-forming units, which become granulocytes and monocytes

These cells have receptors for colony-stimulating factors (CSFs). Each CSF stimulates a different WBC type to develop in response to specific needs. Mature lymphocytes and macrophages secrete several types of CSFs in response to infections and other immune challenges. The red bone marrow stores granulocytes and monocytes until they are needed in the bloodstream. However, circulating leukocytes do not stay in the blood for very long. Granulocytes circulate for 4-8 hours and then migrate into the tissues where they live for another 4-5 days. Monocytes travel in the

blood for 10-20 hours, then migrate into the tissues and transform into a variety of macrophages which can live as long as a few years. Lymphocytes are responsible for long-tern immunity and can survive from a few weeks to decades. They are continually recycled from blood to tissue fluid to lymph and finally back to the blood.

Thrombopoiesis

Thrombopoiesis refers to the production of platelets in the blood, because platelets used to be called thrombocytes. This starts when a haemocytoblast develops receptors for the hormone thrombopoietin which is produced by the liver and kidneys. When these receptors are in place, the haemocytoblast becomes a committed cell called a megakaryoblast. This replicates its DNA, producing a large cell called a megakaryocyte, which breaks up into tiny fragments that enter the bloodstream. About 25-40% of the platelets are stored in the spleen and released as needed. The remainder circulate freely in the blood are live for about 10 days.

Ageing changes in the blood

The properties of blood change as we grow older. It is thought that these changes might contribute to the increased incident of clot formation and atherosclerosis in older people. Some of the most prominent findings on these changes include:

. Rise in fibrinogen
. Rise in blood viscosity
. Rise in plasma viscosity
. Increased red blood cell rigidity
. Increased formation of fibrin degradation products
. Earlier activation of the coagulation system

The increased level of plasma fibrinogen is thought to be due to either its rapid production or slower degradation. As age progresses, fibrinogen and plasma viscosity tend to be positively correlated, with the rise in plasma viscosity being largely attributed to the rise in fibrinogen.

The viscosity of blood depends on factors such as shear rate, haemocrit, red cell deformability, plasma viscosity and red cell aggregation. Although there are many factors involved, hyperviscosity syndrome can be generated by a rise in only one factor. A state of hyperviscosity causes sluggish blood flow and reduced oxygen supply to the tissue.

An age-dependent increase in various coagulation factors, a positive correlation with fibrinogen and a negative correlation with plasma albumin has also been found. Both platelet and red cell aggregation increase with age, with red cell aggregation appearing to be the primary factor responsible for a rise in blood viscosity at low shear rates.

The decrease in red cell deformability (increase in rigidity) refers to its ability to deform under flow forces. Less deformable cells offer more resistance to flow in the microcirculation, which influences the delivery of oxygen to the tissues. Studies have found that older people have less fluid membranes in their red cells.

Blood H+ has also been found to be positively correlated with age, making the blood slightly more acidic as we age. This results in a swelling of the cell, making the red cells less deformable. This sets up a cycle for further increase in blood viscosity and worsening of blood flow parameters.

Since ageing causes a reduction in total body water, blood volume decreases due to less fluid being present in the bloodstream. The number of red blood cells, and the corresponding haemoglobin and haemocrit levels, are reduced which contributes to fatigue in the individual. Most of the white blood cells stay at their original levels, although there is a decrease in lymphocyte number and ability to fight off bacteria, leading to a reduced ability to resist infection.

Overall, the rise in fibrinogen is the most common and significant change in blood during ageing because it contributes to a rise in plasma viscosity, red blood cell aggregation and a rise in blood viscosity at low shear rates. Increased age is associated with a state of hypercoagulation of blood, making older people more susceptible to clot formation and atherosclerosis.

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were first introduced by Wintrobe in 1929 to define the size (MCV) and hemoglobin content (MCH, MCHC) of red blood cells. Termed *red cell indices*, these values are useful in elucidating the etiology of anemias. Red cell indices can be calculated if the values of hemoglobin, hematocrit (packed cell volume), and red blood cell count are known. With the general availability of electronic cell counters, red cell indices are now automatically measured in all blood count determinations.

Variation in the size of red cells (anisocytosis) can be quantified and expressed as red cell distribution width (RDW) or as red cell morphology index. The RDW is more widely available and is discussed in this chapter. The size distribution of a population of cells is graphically represented by the red cell histograms (Price–Jones curves). Similar histograms are also available for white blood cells and platelets.

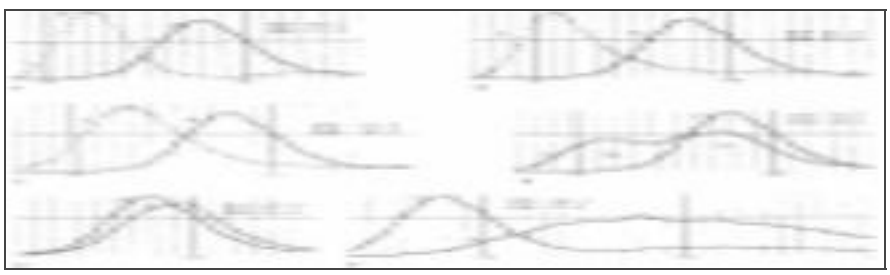


Figure 1

Red cell histograms in various conditions. (A) Heterozygous beta thalassemia. (B) Poor iron utilization (R-E block, chronic disease). (C) Iron deficiency anemia. (D) Dimorphic anemia—iron deficiency, recent transfusion. (E) Macrocytic anemia

MCV defines the size of the red blood cells and is expressed as femtoliters (10−15; fl) or as cubic microns (μm3). The normal values for MCV are 87 ± 7 fl.

MCH quantifies the amount of hemoglobin per red blood cell. The normal values for MCH are 29 ± 2 picograms (pg) per cell.

MCHC indicates the amount of hemoglobin per unit volume. In contrast to MCH, MCHC correlates the hemoglobin content with the volume of the cell. It is expressed as g/dl of red blood cells or as a percentage value. The normal values for MCHC are 34 ± 2 g/dl.

RDW represents the coefficient of variation of the red blood cell volume distribution (size) and is expressed as a percentage. The normal value for RDW is 13 ± 1.5%.

Go to:

Technique

Red cell indices MCV, MCH and MCHC are calculated from hemoglobin, hematocrit, and red blood cell count as follows:

$$\text{MCV} = \frac{\text{Volume of packed cells/ 1000 ml of blood}}{\text{Red blood cell count in millions/ml}} \text{ fl or } \mu m^3$$

$$\text{MCH} = \frac{\text{Hemoglobin in g/ 1000 ml of blood}}{\text{RBC count in millions/ml}} \text{ pg/cell}$$

$$\text{MCHC} = \frac{\text{Hemoglobin in g/ 100 ml of blood} \times 100}{\text{Volume of packed cells/ 100 ml of blood}} \text{ g/dl or \%}$$

Most clinical laboratories now use automated machines to perform blood counts (commonly called CBC) that include red cell indices as part of the profile. Two types of automated machines are generally used. Instruments like the Coulter S model employ the principle of electric impedance; others, like the Hemalog System Analyzer, use optical methods in performing cell counts. Most of the automated machines give the following values: white cell count, red cell count, platelet count, hemoglobin, hematocrit, MCV, MCH, and MCHC. Newer machines, capable of calculating RDW or red cell morphology index, mean platelet volume, absolute lymphocyte count, and differential white cell count ate now being used in many clinical laboratories. These instruments are also capable of producing histograms.

While the automated cell counters are fast, convenient, and precise, certain conditions can interfere with machine calculations and result in spurious values. It is important that clinicians become familiar with the more common causes of spurious results with electronic counters (Table 2):

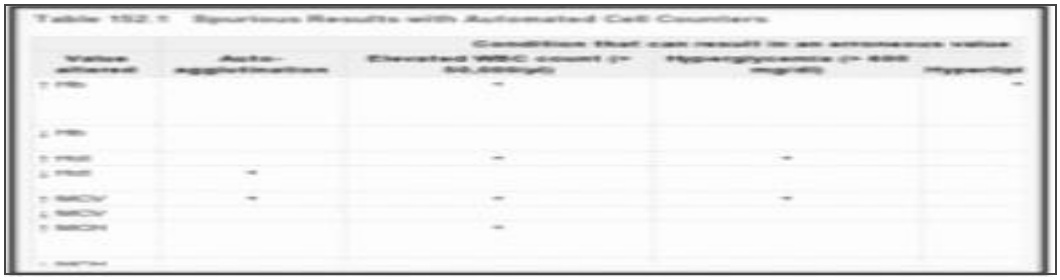


Table 2

Spurious Results with Automated Cell Counters.

In *red cell agglutination*, doublet erythrocytes are counted as one, and larger clumps are not counted as red blood cells at all. This leads to a "decrease" in red cell count and a falsely elevated MCV. Determination of the hemoglobin value is not affected. Prewarming the sample eliminates these spurious values.

In *hyperglycemia*, red cells are transiently hypertonic in relation to the isotonic diluting fluid, resulting in swollen cells and an elevated MCV. This can be avoided if some time is allowed for equilibration after dilution.

Hemoglobin is quantified based on its absorption characteristics. Conditions such as *hyperlipidemias*, hyperbilirubinemia, a very high white blood cell count, and high serum protein can interfere with this measurement and result in falsely elevated hemoglobin values.

Presence of immunoglobulins or fibrinogen precipitated by low temperatures in the blood sample leads to interference with cell counts, resulting in spuriously increased white blood cell count and sometimes small elevations in hemoglobin, hematocrit, red blood cell count, and a slight decrease in MCV. Prewarming the sample to 37°C will correct the artificial values.

When the values of hemoglobin, red cell count, and MCV are affected, MCH and MCHC also become abnormal, since these indices are calculated and are not directly measured.

Sometimes a set of spurious values may be the first clue to an otherwise unsuspected clinical condition (e.g., the combination of low hematocrit, normal hemoglobin, and high MCV and MCHC is characteristic of cold agglutinins). The MCV, since it is an average value, can be normal in the presence of two different cell populations (e.g., dimorphic anemias, red cell fragmentation with reticulocyte response). It is, therefore, important to examine the peripheral smear in the evaluation of anemias. When available, RDW is a good indicator of the degree of anisocytosis. Similarly, the red cell histogram, which offers a graphic depiction of red cell size distribution, will reveal anisocytosis even when the MCV is normal.

Anemia:

During erythropoiesis, the process of erythroid maturation involves a progressive condensation of nuclear chromatin (termed *nuclear maturation*) and finally its extrusion from the cell, the synthesis of hemoglobin in the cytoplasm (termed *cytoplasmic maturation)*, and a concomitant reduction in cell size due to division and water loss.

Defects in nuclear maturation, as seen in megaloblastic anemias due to folate or B12 deficiency, result in large oval erythrocytes (macroovalocytes) with a normal hemoglobin content. The MCV and MCH are increased, while the MCHC remains normal. There is anisocytosis, and RDW is often increased. In the macrocytosis of liver disease, where there is no defect in nuclear maturation, the cells are large due to an excess red cell membrane. These cells are round, rather than oval, and the RDW is normal.

Defective hemoglobin synthesis results in small cells (low MCV) with or without anisocytosis. In heterozygous β-thalassemias, the cells are uniformly small (low MCV; RDW tends to be normal), whereas in iron deficiency, anisocytosis (increased RDW) may be the first laboratory abnormality, even before anemia and microcytosis are seen.

In abnormalities involving nuclear maturation, hemoglobin production proceeds normally, while cell division lags behind, ultimately leading to a larger than normal cell. In contrast, when there is defective and delayed synthesis of hemoglobin, the continued cell division leads to microcytosis.

Clinical Significance

Anemias may be classified based on their etiology (e.g., hemolytic, hemorrhagic, etc.), erythropoietic response (e.g., hypoproliferative, ineffective), or cell morphology (e.g., macrocytic, microcytic-hypochromic).

Red cell indices are valuable in the morphologic classification of anemias. Since different etiologic factors result in characteristically different red cell morphology, the clinician can properly plan the management of a patient with an anemia if he can interpret the blood counts and peripheral blood smear well.

Anemias are classified, according to the size of the red cell, as being normocytic (normal MCV), macrocytic (increased MCV), or microcytic (decreased MCV). Microcytic anemias were also often described as being hypochromic based on peripheral smear examination and MCHC when this value was determined manually. MCHC as measured by the electronic machines is mostly normal in microcytic anemias, however, and the value of MCH closely parallels the value of MCV. The optical properties of the small, thin microcytes make them appear hypochromic on the blood smear, while the hemoglobin concentration remains in the normal range (microcytic, normochromic anemias).

There are no hyperchromic anemias. In spherocytosis, the MCHC is increased due to loss of membrane and the consequent spherical shape assumed by the cell.

The general availability of RDW as a measure of anisocytosis helps further in the evaluation of anemias based on morphology (see Table 2). Significant anisocytosis often leads to an increased RDW, whereas in its absence the RDW remains normal.

Table 2

MCV and RDW in the Evaluation of Anemias.

It should be pointed out again that *an evaluation of anemia*s *is not complete without the careful examination of a well-prepared peripheral blood smear.* Red cell indices, RDW, and red blood cell histograms will not help identify conditions such as red cell inclusions (e.g., malarial parasites) or membrane abnormalities such as spherocytosis that might be responsible for the anemia.

Clotting is a process in which liquid blood is converted into a gelatinous substance that eventually hardens. The aim is to stop the flow of blood from a vessel. The formation of a clot is the result of a series of enzymatic reactions that are triggered upon injury. The process involves:

1. a step of activation (wounding) followed by
2. a cellular response (aggregation of blood platelets) and
3. a molecular response (polymerization of the protein called fibrin to create a meshwork that hardens).

Factors released in the cellular response help activate the molecular response. The process is highly conserved across species.

Cellular Response

Injury to the epithelial lining of a blood vessel begins the process of coagulation almost instantly. The cellular response has an initial action followed by an amplification step. In the cellular response (Figure 4.68), the platelets bind directly to collagen using Ia/IIa collagen-binding surface receptors and glycoprotein VI to form a plug. The signal to the platelets to take this action is exposure of the underlying collagen, something that would not happen in the absence of a wound. Upon injury, platelet integrins get activated and bind tightly to the extracellular matrix to anchor them to the site of the wound.

The von Willebrand factor (see below also) assists by forming additional links between the platelets' glycoprotein Ib/IX/V and the fibrils of the collagen.

Amplification

In the amplification part of the cellular response, the activated platelets release a large number of factors, including platelet factor 4 (a cytokine stimulating inflammation

and moderating action of the heparin anticoagulant) and thromboxane A2, The latter has the effect of increasing the "stickiness" of platelets, favoring their aggregation. In addition, a a Gq-protein linked receptor cascade is activated, resulting in release of calcium from intracellular stores. This will play a role in the molecular response.

Molecular response

The molecular response results in the creation of a web comprised of polymers of fibrin protein. Like the cellular pathway, the molecular pathway begins with an initiation phase and continues with an amplification phase. Polymerization of fibrin results from convergence of two cascading catalytic pathways. They are the intrinsic pathway (also called the contact activation pathway) and the extrinsic pathway (also referred to as the tissue factor pathway). Of the two pathways, the tissue factor pathway has recently been shown to be the more important.

Serine Protease Cascade

In both pathways, a series of zymogens of serine proteases are sequentially activated in rapid succession. The advantage of such a cascading system is tremendous amplification of a small signal. At each step of the cascade, activation of a zymogen causes the production of a considerable amount of an active serine protease, which is then able to activate the next zymogen which, in turn, activates an even larger amount of the next zymogen in the system. This results in the ultimate activation of a tremendous amount more fibrin than could be achieved if there were only a single step where an enzyme activated fibrinogen to fibrin.

Nomenclature

The zymogen factors in the molecular response are generally labeled with Roman numerals. A lowercase, subscripted 'a' is used to designate an activated form.

The tissue factor pathway functions to create a thrombin burst, a process in which thrombin is activated very quickly. This is the initiation phase. It is fairly straightforward because it has one focus - activation of thrombin. Thrombin, which converts fibrinogen into the fibrin of the clot, is central also to the amplification phase, because it activates some of the factors that activate it, creating an enormous increase in signal and making a lot of thrombin active at once.

Initiation phase

The initiation phase of the molecular response begins when Factor VII (the letter 'F' before the Roman numeral is often used as an abbreviation for 'factor') gets activated to FVIIa after damage to the blood vessel (Figure 4.69 & 4.70). This happens as a result of its interaction with Tissue Factor (TF, also called coagulation Factor III) to make a TF-FVIIa complex. The combined efforts of TF-FVIIa, FIXa, and calcium (from the cellular response) inefficiently convert FX to FXa. FXa, FV, and calcium inefficiently convert prothrombin (zymogen) to thrombin (active). A tiny amount of thrombin has been activated at the end of the initiation phase.

Amplification phase

To make sufficient thrombin to convert enough fibrinogen to fibrin to make a clot, thrombin activates other factors (FV, FXI, FVIII) that help to make more thrombin. This is the amplification phase of the molecular process and is shown in the light blue portion in the upper right part. The amplification phase includes factors in both the intrinsic and extrinsic pathways. FVIII is normally bound in a complex with the von Willebrand factor and is inactive until it is released by action of thrombin. Activation of FXI to FXIa helps favor production of more FIXa. FIXa plus FVIIIa stimulate production of a considerable amount of FXa. FVa joins FXa and calcium to make a much larger amount of thrombin. Factors FVa and FVIIIa are critical to the amplification process. FVIIIa stimulates FIXa's production of FXa by 3-4 orders of magnitude. FVa helps to stimulate FXa's production of thrombin by about the same magnitude. Thus, thrombin stimulates activation of factors that, in turn, stimulate activation of more thrombin.

Transglutaminase

In addition to helping to amplify product of itself and conversion of fibrinogen to fibrin, thrombin catalyzes the activation of FXIII to FXIIIa. FXIIIa is a transglutaminase that helps to "harden" the clot (Figure 4.71 & 4.73). It accomplishes this by catalyzing formation of a covalent bond between adjacent glutamine and lysine side chains in the fibrin polymers.

Not all of the factors involved in the clotting process are activated by the pathway, nor are all factors serine proteases. This includes FVIII and FV which are glycoproteins, and FXIII, which is the transglutaminase described above.

The blood clotting process must be tightly regulated. Formation of clots in places where no damage has occurred can lead to internal clots (thrombosis) cutting off the flow of blood to critical regions of the body, such as heart or brain. Conversely, lack of clotting can lead to internal bleeding or, in severe cases, death due to unregulated external bleeding. Such is a danger for people suffering from hemophilia.

Diseases of Blood Clotting: Hemophilia

Hemophilia is a hereditary genetic disorder affecting the blood clotting process in afflicted individuals. The disease is X-linked and thus occurs much more commonly in males. Deficiency of FVIII leads to Hemophilia A (about 1 in 5000 to 10,000 male births) and deficiency of FIX produces Hemophilia B (about 1 in 20,000 to 35,000 male births).

Hemophilia B spread through the royal families of Europe, beginning with Queen Victoria's son, Leopold. Three of the queen's grandsons and six of her great-grandsons suffered from the disease. Hemophilia is treated by exogenous provision of missing clotting factors and has improved life expectancy dramatically. In 1960, the life expectancy of a hemophiliac was about 11 years. Today, it is over 60.

Queen Victoria, whose descendants suffered from hemophilia B

Diseases of Blood Clotting: von Willebrand's disease

A related disease to hemophilia that is also genetically linked is von Willebrand's Disease. The von Willebrand factor plays a role in both the cellular and the molecular responses in blood clotting. First, the factor is a large multimeric glycoprotein present in blood plasma and also is produced in the endothelium lining blood vessels.

The von Willebrand factor helps to anchor platelets near the site of the wound in the cellular response. It binds to several things. First, it binds to platelets' Ib glycoprotein. Second, it binds to heparin and helps moderate its action. Third, it binds to collagen and fourth, the factor binds to FVIII in the molecular response, playing a protective role for it. In the absence of the von Willebrand factor, FVIII is destroyed. Fifth, the von Willebrand factor binds to integrin of platelets, helping them to adhere together and form a plug. Defects of the von Willebrand factor lead to various various bleeding disorders.

Blood "thinners"

The clotting of blood is essential for surviving wounds that cause blood loss. However, some people have conditions that predispose them to the formation of clots that can lead to stroke, heart attack, or other problems, like *pulmonary embolism*. For these people, anti-clotting agents (commonly called blood thinners) are used to reduce the likelihood of undesired clotting.

The first, and more common of these is aspirin. Aspirin is an inhibitor of the production of prostaglandins. Prostaglandins are molecules with 20 carbons derived from arachidonic acid that have numerous physiological effects. Metabolically, the prostaglandins are precursors of a class of molecules called the thromboxanes. Thromboxanes play roles in helping platelets to stick together in the cellular response to clotting. By inhibiting the production of prostaglandins, aspirin reduces the production of thromboxanes and reduces platelet stickiness and the likelihood of clotting.

Vitamin K action

Another approach to preventing blood clotting is one that interferes with an important molecular action of Vitamin K. A pro-clotting factor found in the blood, vitamin K is necessary for an important modification to prothrombin and other blood clotting proteins. Vitamin K serves as an enzyme cofactor that helps to catalyze addition of an extra carboxyl group onto the side chain of glutamic acid residues of several clotting

enzymes. This modification gives them the ability to bind to calcium.which is important for activating the serine protease cascade. During the reaction that adds carboxyl groups to glutamate, the reduced form of vitamin K becomes oxidized. In order for vitamin K to stimulate additional carboxylation reactions to occur, the oxidized form of vitamin K must be reduced by the enzyme vitamin K epoxide reductase.

Warfarin blocks reduction

The compound known as warfarin (brand name = coumadin - Figure 4.78) interferes with the action of vitamin K epoxide reductase and thus, blocks recycling of vitamin K. As a consequence, fewer prothrombins (and other blood clotting proteins) get carboxylated, and less clotting occurs.

Vitamin K-mediated carboxylation of glutamate occurs on the γ carbon of the amino acid's side chain, for 16 different proteins, 7 of which are involved in blood clotting, including prothrombin. When the carboxyl group is added as described, the side chain is able to efficiently bind to calcium ions. In the absence of the carboxyl group, the side chain will not bind to calcium. Calcium released near the site of the wound in the cellular response to clotting helps to stimulate activation of proteins in the serine protease cascade of the molecular response.

Vitamin K comes in several forms. It is best described chemically as a group of 2-methyl-1,4-naphthoquinone derivatives. There are five different forms recognized as vitamin Ks (K1, K2, K3, K4, and K5). Of these, vitamins K1 and K2 come from natural sources and the others are synthetic. Vitamin K2, which is made from vitamin K1 by gut microorganisms, has several forms, with differing lengths of of isoprenoid side-chains. The various forms are commonly named as MK-X, where X is a number, and MK stands for menaquinone, which is the name given to this form of vitamin K. Figure 4.79 shows a common form known as MK-4 (menatetrenone).

Hemorrhaging danger

It is very critical that the proper amount of warfarin be given to patients. Too much can result in hemorrhaging. Patients must have their clotting times checked regularly to ensure that they are taking the right dose of anti-coagulant medication. Diet and the metabolism of Vitamin K in the body can affect the amount of warfarin needed. Vitamin K is synthesized in plants and plays a role in photosynthesis. It can be found in the highest quantities in vegetables that are green and leafy. Patients whose diet is high in these vegetables may require a different dose than those who rarely eat greens. Dietary vitamin K is also, as mentioned earlier, metabolized by bacteria in the large intestine, where they convert vitamin K1 into vitamin K2.

Plasmin

Clots, once made in the body, do not remain there forever. Instead, a tightly regulated enzyme known as plasmin is activated, when appropriate, to break down the fibrin-entangled clot. Like many of the enzymes in the blood clotting cascade, plasmin is a serine protease. It is capable of cleaving a wide range of proteins. They include polymerized fibrin clots, fibronectin, thrombospondin, laminin, and the von Willebrand factor.

Plasmin plays a role in activating collagenases and in the process of ovulation by weakening the wall of the Graafian follicle in the ovary. Plasmin is made in the liver as the zymogen known as plasminogen. Several different enzymes can activate it.

Tissue plasminogen activator (tPA), using fibrin as a co-factor, is one. Others include urokinase plasminogen activator (using urokinase plasminogen activator receptor as a co-factor), kallikrein (plasma serine protease with many forms and blood functions), and FXIa and FXIIa from the clotting cascade.

Plasmin inhibition

Plasmin's activity can also be inhibited. Plasminogen activator inhibitor, for example, can inactivate tPA and urokinase. After plasmin has been activated, it can also be inhibited by α2-antiplasmin and α2-macroglobulin (Figure 4.80). Thrombin also plays a role in plasmin's inactivation, stimulating activity of thrombin activatable fibrinolysis inhibitor. Angiostatin is a sub-domain of plasmin produced by auto-proteolytic cleavage. It blocks the growth of new blood vessels and is being investigated for its anti-cancer properties.

Fibronectin

Fibronectin is a large (440 kDa) glycoprotein found in the extracellular matrix that binds to integral cellular proteins called integrins and to extracellular proteins, including collagen, fibrin, and heparan sulfate. It comes in two forms. The soluble form is found in blood plasma and is made by the liver. It is found in high concentration in the blood stream (300 μg/ml). The insoluble form is found abundantly in the extracellular matrix.

The protein is assembled in the extracellular matrix and plays roles in cellular growth, adhesion, migration, and differentiation. It is very important in wound healing.

Assists in blood clot formation

Fibronectin from the blood plasma is localized to the site of the wound, assisting in formation of the blood clot to stop bleeding. In the initial stages of wound healing, plasma fibronectin interacts with fibrin in clot formation. It also protects tissue surrounding the wound. Later in the repair process, remodeling of the damaged area begins with the action of fibroblasts and endothelial cells at the wound site. Their task is to degrade proteins of the blood clot matrix, replacing them with a new matrix like the undamaged, surrounding tissue.

Fibroblasts act on the temporary fibronectin-fibrin matrix, remodeling it to replace the plasma fibronectin with cellular fibronectin. This may cause the phenomenon of

wound contraction, one of the steps in wound healing. Secretion of cellular fibronectin by fibroblasts is followed by fibronectin assembly and integration with the extracellular matrix.

Embryogenesis

Fibronectin is essential for embryogenesis. Deleting the gene in mice causes lethality before birth. This is likely due to its role in migration and guiding the attachment of cells as the embryo develops. Fibronectin also has a role in the mouth. It is found in saliva and is thought to inhibit colonization of the mouth by pathogenic bacteria.

Platelet activating factor

Platelet Activating Factor (PAF) is a compound (Figure 4.83) produced primarily in cells involved in host defense. These include platelets, macrophages, neutrophils, and monocytes, among others. It is produced in greater quantities in inflammatory cells upon proper stimulation. The compound acts like a hormone and mediates platelet aggregation/degranulation, inflammation, and anaphylaxis. It can transmit signals between cells to trigger and amplify inflammatory and clotting cascades.

When unregulated, signaling by PAF can cause severe inflammation resulting in sepsis and injury. Inflammation in allergic reactions arises partly as a result of PAF and is an important factor in bronchoconstriction in asthma. In fact, at a concentration of only 10 picomolar, PAF can cause asthmatic inflammation of the airways that is life threatening.

BLEEDING TIME

The bleeding time test is dependent on appropriate functioning of platelets blood vessels and platelets and evaluates earliest hemostasis (platelets components and vascular). In this test, incision (a surgical cut made in skin) or a superficial skin puncture is made and the time is measured for bleeding to stop. There are three methods most commonly used to measure bleeding time:

Ivy's Method Principle: On the volar surface of forearm, three normal punctures are made with the help of a lancet under normal pulse pressure (between 30-40 mm Hg). The average time is measured for bleeding to stop from the puncture sites.

Equipment 1. Disposable sterile lancets 2. Sphygmomanometer 3. Filter paper 4. Stopwatch Method

1. Blood pressure of the patient is measured with the help of sphygmomanometer. The blood pressure of the patient should be normal before going to the further process.
2. The volar surface of the forearm is cleansed with ethanol 70% and allowed to dry.
3.With the help of a lancet, in quick succession, three punctures are made about 5 cm apart. Note that scars and superficial veins should be avoided.
4. Start the stopwatch as soon as puncture made on the volar surface of the forearm.
5.With the help of the filter paper, blood oozing from the puncture wound is gently absorbed with intervals of 15 seconds.
6. The timer is stopped when blood no more mark the filter paper.
7. Time measured for bleeding to stop from all the three puncture wound is recorded. The average time is calculated and reported as the bleeding time.

Reference Ranges • Normal range: 2 -7 minutes.
• The greater numbers of individuals have bleeding time less than 4 minutes. The bleeding time should be reported in minutes or nearest half minute. If the bleeding continues more than twenty minutes, the test is stopped and the bleeding time should be reported as >20 minutes (more than 20 minutes). Cause of extend of duration of bleeding time

1. Disorders of blood vessels
2. Thrombocytopenia: This term is uses when the platelet count is less than its normal value. The bleeding time test should not be performed the platelet count is less than 1,00,000/ml. It may be difficult to control the bleeding if the platelet count is very low.
3. Von Willebrand disease.4. Disorder of platelet function
4. Afibrinogenemia

CLOTTING TIME
In this test, required time is measured for the blood to clot in a glass test tube, kept at 37° C. Extend of duration of clotting time occurs only if severe deficiency of a clotting factor exists and is normal in moderate or mild deficiency.
PRINCIPLE Tissue thromboplastin, in the presence of calcium ions and Factor VII, activates the extrinsic pathway of coagulation. When a mixture of tissue thromboplastin and calcium ions is added to normal anticoagulant plasma, the clotting mechanism is initiated and a clot will form within a specified time period. If a deficiency exists within the extrinsic pathway, the time required for clot formation will be prolonged. The degree of prolongation is proportional to the severity of a single factor deficiency, or a cumulative deficiency of all the factors involved.
MATERIALS PT Reagent: 1.0 Plasma Normal and Abnormal Controls (Optional).

SPECIMEN COLLECTION AND PREPARATION 1. A sample of the patient's blood is obtained by venipuncture in a tube with 0.109M sodium citrate as an anticoagulant at a 9:1 ratio. Centrifuge the whole blood specimen at 2500xg for 15 minutes. Aspirate the plasma using a plastic pipette and place it in a plastic test tube. Perform the Prothrombin Time assay within 4 hours. 2. Reconstitute the normal control plasma and abnormal control plasma according to the package insert included within.
PROCEDURE
A. Manual Method

1. Bring all reagents, controls and sample to room temperature. 2. Pre-warm PT reagent at 37°C for 20 minutes. 3. Pipette 100 µl of PT reagent into tube. 4. Pre-warm samples and controls at 37°C for 3 minutes. 5. Add 50 µl of sample or controls to labeled containing the PT reagent tubes, start stop watch and mix in a water bath/ 37°C. 6. Record the time required for clot formation.

RESULTS Prothrombin Time Ratio (PTR) = Clot time of the test plasma Clot time of the control plasma INR = PTRISI Example: for a PTR of 2.0 and an ISI of 1.0 INR = 2.01.0 = 2.0 REFERENCE VALUES INR: 0.8-1.24
1. These values should only serve as guidelines. Because differences may exist between instruments, laboratories, and local populations, it is recommended that each laboratory establish its own reference range of expected Prothrombin time results.

2.The results of the Prothrombin Time tests should be reported to the nearest tenth of a second. Results greater than the upper limits of the range should be considered abnormal and follow-up testing should be performed. PT values below the lower limits of the range may indicate a compromised sample, and a new sample should be collected.
Table for INR values for PT Ratios between (0.05 - 6.0)

ISI Value = 1.0 PTR INR PTR INR PTR INR 0.05 0.05 2.05 2.05 4.05 4.05 0.10 0.10 2.10 2.10 4.10 4.10 0.15 0.15 2.15 2.15 4.15 4.15 0.20 0.20 2.20 2.20 4.20 4.20 0.25 0.25 2.25 2.25 4.25 4.25 0.30 0.30 2.30 2.30 4.30 4.30 0.35 0.35 2.35 2.35 4.35 4.35 0.40 0.40 2.40 2.40 4.40 4.40 0.45 0.45 2.45 2.45 4.45 4.45 0.50 0.50 2.50 2.50 4.50 4.50 0.55 0.55 2.55 2.55 4.55 4.55 0.60 0.60 2.60 2.60 4.60 4.60 0.65 0.65 2.65 2.65 4.65 4.65 0.70 0.70 2.70 2.70 4.70 4.70 0.75 0.75 2.75 2.75 4.75 4.75 0.80 0.80 2.80 2.80 4.80 4.80 0.85 0.85 2.85 2.85 4.85 4.85 0.90 0.90 2.90 2.90 4.90 4.90 0.95 0.95 2.95 2.95 4.95 4.95 1.00 1.00 3.00 3.00 5.00 5.00 1.05 1.05 3.05 3.05 5.05 5.05 1.10 1.10 3.10 3.10 5.10 5.10 1.15 1.15 3.15 3.15 5.15 5.15 1.20 1.20 3.20 3.20 5.20 5.20 1.25 1.25 3.25 3.25 5.25 5.25 1.30 1.30 3.30 3.30 5.30 5.30 1.35 1.35 3.35 3.35 5.35 5.35 1.40 1.40 3.40 3.40 5.40 5.40 1.45 1.45 3.45 3.45 5.45 5.45 1.50 1.50 3.50 3.50 5.50 5.50 1.55 1.55 3.55 3.55 5.55 5.55 1.60 1.60 3.60 3.60 5.60 5.60 1.65 1.65 3.65 3.65 5.65 5.65 1.70 1.70 3.70 3.70 5.70 5.70 1.75 1.75 3.75 3.75 5.75 5.75 1.80 1.80 3.80 3.80 5.80 5.80 1.85 1.85 3.85 3.85 5.85 5.85 1.90 1.90 3.90 3.90 5.90 5.90 1.95 1.95 3.95 3.95 5.95 5.95 2.00 2.00 4.00 4.00 6.00 6.00

Blood Smear and WBC Count

Aim: Learn to count cells, observe and identify different blood cells in a smear, quantify their proportions and count RBCs per µl (mm^3) using a haemocytometer.
Introduction: The technique of making a peripheral blood wedge slide (or push slide) was developed by Maxwell Wintrobe1. Hematology is the study of blood and the blood smear one of the most basic and yet most reliable ways of evaluating blood for multiple conditions of disease. We will use a droplet of blood to make a thin smear, dry it, fix and stain it and observe under a microscope. Cell fixation is done by placing the slide in methanol (CH3OH). It works by precipitating proteins and carbohydrates. Additionally it also dehydrates the sample. The action on lipids is thought to involve dissolution. It is expected by dehydration the cells undergo some amount of shrinkage. Staining by a combination of acidic (cytoplasm label) and basic (DNA label) dyes leads to very good contrast images of not just red blood cells (RBCs) but also white blood cells (WBCs) of various types.
The Hemocytometer is a classic device used to measure cell numbers, particularly in blood samples. Counting is performed by introducing citrated (4% w/v sodium citrate (dihydrate), pH adjusted with citric acid, USP) blood into the counting region of the chamber (Figure 1). The height of the chamber is 0.1 mm. Using this we can estimate the volume occupied in the boxes marked R (for RBC). The total length of one side of 5 R-boxes is 1 mm. Using this measure and a mean count of cells in each R-box, we can estimate the number of RBC's in a unit of blood as follows:
CRBC = NRBC R−box · 25 VR25 · df (Eq. 1),
CRBC= RBC count (cells/µl) NRBC R−box = Mean RBC-count from five R-boxes (usually the 4-corners and central) VR25 = Vol. of the 5x5 RBC region in µl df =Dilution factor. This count has been shown to vary between men and women. We will take one sample each to test this. Counting is done by eye. 1 Wintrobe MM.

(1932) The size and hemoglobin content of the erythrocyte.When counting certain conventions need to be used. Cells at edges of a line are counted only in the L-shape, i.e. lower line and left-lines. This reduces overcounting artefacts. Averaging over 4-5 R-boxes ensures inhomogeneities in spreading or clumping of cells don't affect the final result. Figure 1: Top view of the chambers of a Neubauer's Hemocytometer for RBC (R) and WBC (W) counting with scales indicating sizes of each region. The height in the z-direction of the entire chamber is uniformly 0.1 mm.

Materials: Biologicals:Droplet of blood from capillary bleed Glass/plastic ware:

1. RBC diluting pipette with hose and bulb
2. 50 ml beaker for waste material Trash bin for lancets
3. Plastic droppers

Chemicals

1. RBC diluent 3.2 or 4% w/v Sodium Citrate ($Na_3C_6H_5O_7$)
2. A 2.5% bleach mixture for cleaning
3. 95% Alcohol for rinsing
4. 99% Methanol for fixation
5. Giemsa stain (1:20, vol/vol from stock)

Instruments 1. Sterile lancet 2. Hemocytometer for RBC and WBC counting 3. Microscope Others 1. Tissue paper 2. Gloves for use while staining

DIFFERENTIAL COUNT:

METHOD A. Making blood smear

1. Place a clean slide on a piece of tissue paper and write your unique initials in one corner, using the glass-marker pen. 2. Using a cotton swab dipped in 75% ethanol, clean the middle- or ring-finger with it. This both disinfects the surface as well as causes a slight increase in blood flow due to the evaporation and resultant compensatory blood-rush. 3. Carefully open the protective covering of a sterile Stab gently finger-tip and wait for a drop of blood to come up. 4. Make this drop fall 1 cm from one of the short-edges of a clean slide. 5. Using the other slide, make contact at 30-40 degrees with the slide on which the blood droplet is. Drag the droplet towards the other end. As a result you should have a comet-like appearance of the blood smear. 6. The smear should have a 'comet' like appearance- thick initially and becoming very thin at the end. The comet tail area is the one we will observe under the microscope.

B. Staining the smear

1. Fix the smear in ~99% methanol 3-5 minutes by dipping in Coplin Jars. 2. Stain in Giemsa (methylene blue and eosin mixture) by dipping in Coplin Jar containing stain for a total time ~15 minutes. 3. Rinse the slide with tap water at room temperature (ensure rinsing doesn't wash away your sample). 4. Drain off the water by leaning it at ~45 degrees and leave it to air-dry.

C. Microscopy

Observe under 10x magnification to see that the cells are stained. Move to the 40x lens to see further details (be careful to prevent lens and slide collisions). Erythrocytes are the most numerous cells with a diameter of ~6-7 micrometers. Some larger macrocytes (d>9 um) and smaller microcytes

MANUAL PLATELET COUNT

Principle: Whole blood is diluted with a 1% ammonium oxalate solution. The isotonic balance of the diluent is such that all erythrocytes are lysed while the leukocytes, platelets, and reticulocytes remain intact.1,2 The standard dilution for

platelet counts is 1:100. This dilution is prepared using the leukocyte/platelet Unopette system
1 The dilution is mixed well and incubated to permit lysis of the erythrocytes. Following the incubation period, the dilution is mounted on a hemacytometer. The cells are allowed to settle and then are counted in a specific area of the hemacytometer chamber under the microscope. The number of platelets is calculated per µL (x 109 /L) of blood. Reagents and Equipment 1. Two leukocyte/platelet Unopette reservoirs; each containing 1.98 mL of the following diluent: Ammonium oxalate 11.45 g Sorensen's phosphate buffer 1.0 g Thimerosal 0.1 g QS with distilled water to 1 liter 2. Two Unopette capillary pipets, 20 µL 3. Hemacytometer with cover glass 4. Petri dish with filter paper 5. Hand counter 6. Microscope Quality Control Commercial quality control materials with established control limits should be run periodically. The frequency is determined by each laboratory's workload. For instance, quality control material may be run at the beginning of each eight-hour shift. Specimen Whole blood, anticoagulated with EDTA, or free-flowing capillary blood may be used. Procedure 1. Prepare two leukocyte/platelet Unopettes as follows: a. Using the protective shield on the capillary pipet, puncture the diaphragm as follows: 1) Place reservoir on a flat surface. Grasping the reservoir in one hand, take the pipet assembly in the other hand and push the tip of the pipet shield firmly through the diaphragm in the neck of the reservoir, then remove. b. Remove the shield from the pipet assembly with a twist and fill the capillary pipet with whole blood. Transfer the whole blood to reservoir as follows: 1) Wipe excess blood from the outside of the capillary pipet, making certain that no blood is removed from the capillary bore. 2) Squeeze the reservoir slightly to force out some air. Maintain pressure on the reservoir. 3) Cover opening of overflow chamber of the pipet with your index finger and seat the pipet securely in the reservoir neck. 4) Release pressure on the reservoir. Then remove your finger from the pipet opening. Negative pressure will draw the blood into the diluent. 5) Squeeze the reservoir gently two or three times to rinse the capillary bore, forcing diluent into, but not out of, the overflow chamber, releasing pressure each time to return the mixture to the reservoir. 6) Place your index finger over the pipet opening and gently invert several times to thoroughly mix the blood with diluent. 7) Let stand for 10 minutes to allow erythrocytes to hemolyze. 2. Clean the hemacytometer and cover glass by flooding them with 70% alcohol. Dry thoroughly with gauze or tissue; do not allow the alcohol to dry on the hemacytometer. Be sure to remove all lint. Place the cover glass in position over the ruled area. 3. Following incubation, mix diluted blood thoroughly by inverting reservoir to resuspend cells. Charge hemacytometer as follows: a. Convert to dropper assembly by withdrawing the pipet from the reservoir and reseating it securely in its reverse position. b. Clean the capillary bore by inverting the reservoir, gently squeeze the sides, and discard the first three or four drops. c. Place the pipet tip on the edge of the ruled area of the counting chamber. Carefully charge the hemacytometer with diluted blood by gently squeezing the sides of the reservoir to expel the contents until the chamber is properly filled. d. Repeat procedure to charge the other side of the hemacytometer with the first Unopette reservoir. e. Place the hemacytometer on moistened filter paper in a Petri dish, and allow to stand 10 minutes to permit the cells to settle. f. Using this same procedure, charge a second hemacytometer with the second Unopette reservoir. 4. Carefully place the hemacytometer on the microscope stage. Perform cell count as follows: a. With the low-power (10x) objective, locate the ruled area and the center large square (1 mm2). Examine the entire center square for even distribution of

platelets, then carefully switch to the high-dry-power (40x) phase objective for counting platelets. With phase microscopy, platelets appear as round or oval bodies. b. Platelets are counted in the entire center large square (1 mm2)

1) Count the platelets in the first row of squares going from left to right, then from right to left in the second row; follow this pattern until all rows are counted.

2) Within each square, count all platelets touching the top and left-hand borders. Do not count any cells touching the bottom or right-hand borders.

3) Use the fine adjustment knob to focus up and down to identify the platelets. c. Repeat this counting procedure for the other side of the hemacytometer. d. Record the counts for each center square. The difference between these two counts should not exceed 10%. e. Count the platelets on the second hemacytometer following the above counting procedure. Calculations 1. The calculation formula for hemacytometer cell counts determines the number of cells within 1 µL (1 mm3) of blood (Figure 7-9). To make this determination, the total number of cells counted must be corrected for the initial dilution of blood and the volume of diluted blood used. The standard dilution of blood for platelet counts is 1:100; therefore the dilution factor is 100. The volume of diluted blood used is based on the area and depth of the counting area. The area counted is 2 mm2 and the depth is 0.1 mm; therefore the volume factor is 0.2 mm3 . Total number of cells counted • dilution factor • 1/volume factor = cells/mm3 Cells/mm3 = cells/µL or cells/µL • 103 µL /L = cells x 109 /L Example: 100 x 103 platelets/µL • 103 µL/L = 100 x 109 platelets/L 2. Average the platelet counts from the duplicate pipets and report result (x 109/L or /mm3). Reference Interval 150-440 x 109 /L Comments 1. Platelet counts should be performed within three hours after the dilution has been prepared. 2. The coefficient of variation (CV) for the 95% confidence limits is + 22%. 3. If blood is collected by a skin puncture, carefully remove the first drop of blood and collect free-flowing blood for the platelet count. This will minimize the occurrence of platelet clumping and adhesion of platelets to the puncture site. 4. If clumps of platelets are seen in the hemacytometer, the procedure should be repeated. Clumps may be due to inadequate mixing of blood or to poor technique in obtaining the blood specimen. 5. A Wright's-stained peripheral blood smear should be examined and the platelet estimate determined to confirm the hemacytometer platelet count. The platelet estimate should correlate with the platelet count + 25%. If a discrepancy exists, the platelet count and peripheral blood smear estimate should be repeated. 6. In acute leukemia, there is an increase of blast cells in the peripheral blood. Often fragments of cytoplasm about the size of platelets break off the blast cells. These cytoplasmic fragments are called hyaline bodies. They are the same size and density as platelets and may be counted as platelets by the hemacytometer or automated methods. It is very important that all platelet counts be confirmed by slide examination so that a false increase of platelets (due to the counting of fragments) is not reported. 7. Platelet satellitism will result in falsely decreased platelet counts. With platelet satellitism, platelets adhere to neutrophils when the blood sample is anticoagulated with EDTA and is not free to be counted. Platelet satellitism can be corrected by redrawing the blood sample using sodium citrate as the anticoagulant. The resulting platelet count must be multiplied by 1.1 to account for the dilutional effect of the citrate anticoagulant. 8. A second method for manual platelet counts is the Rees Ecker method. Using the erythrocyte diluting pipet, whole blood is diluted with a solution containing brilliant cresyl blue, which stains the platelets a light bluish color. The platelets are then counted using a standard hemacytometer and bright field microscopy. 9. Technical sources of error in hemacytometer cell counts.

HEMOGLOBIN ESTIMATION

Hemoglobin is a conjugated protein that comprises of 90% of dry weight of RBC

Hemoglobin is tetramer containing two pairs of similar polypeptide chains called globin chains. To each of the four chains is attached heme which is complex of iron in ferrous form and protoporphyrin.

Type of hemoglobin	Structure	Age at which they are present
Adult hemoglobin (HbA)	$\alpha_2\beta_2$	Adults
Hemoglobin A_2	$\alpha_2\delta_2$	Very small amount (3%) in adults.
Fetal hemoglobin (HbF)	$\alpha_2\gamma_2$	Fetal life
Hb Portland	$\zeta_2\gamma_2$	Embryonic life
Gower - 1	$\zeta_2\varepsilon_2$	Embryonic life
Gower – 2	$\alpha_2\varepsilon_2$	Embryonic life
Hemoglobin Barts	γ_4	Fetal life. Increases in thalassemia

Types of hemoglobins

Abnormal hemoglobins – Hb S, Hb C, Hb D, Hb E

Functions of hemoglobin

Carries oxygen to tissues

Carries carbon dioxide from tissues to lungs

Normal hemoglobin concentration

Men – 13 – 18g/dl

Women – 12 – 16g/dl

New born – 16 – 22 g/dl

Children – 12 – 14g/dl

WHO cut off value below which anemia is present

Men – <13g/dl

Women – <12g/dl

Pregnant – <11g/dl

INDICATIONS FOR HEMOGLOBIN ESTIMATION

Detremine the presence and severity of anemia

Screening for polycythemia

To assess response to specific therapy in anemia

Estimation of Red cell indices

Selection of blood donors

METHOD OF COLLECTION OF BLOOD FOR HEMOGLOBIN ESTIMATION

Finger prick (in children and adults)

From veins (venous blood)

Blood collected in EDTA (1.0 to 1.2mg/ml) or double oxalate (2mg/1ml) in appropriate proportion (Double oxalate is mixture of 3 parts of ammonium oxalate and 2 parts of potassium oxalate)

Heal prick (in infants)

SAHLI'S ACID HEMATIN METHOD

Principle

N/10 HCl converts hemoglobin into soluble unstable acid hematin. The colour intensity of the acid hematin after dilution is compared with standard brown glass in the comparator

Apparatus required

Sahli's hemoglobinometer

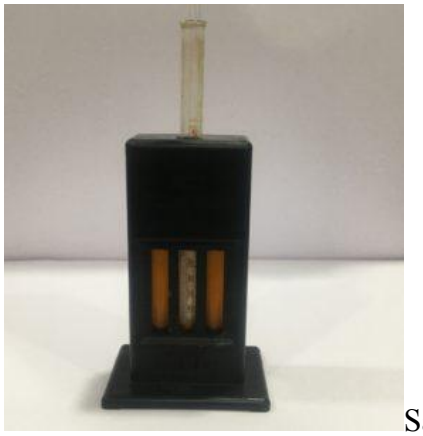
It contains hemoglobin tube, comparator, hemoglobin pipette and stirrer

Hemoglobin pipette – pipette has one mark indicating 20cumm (no bulb)

Hemoglobin tube – it is graduated on both sides. One side is graduated in gram percentage from 2 – 22 (g%). Other side is graduated as percentage from 20 – 140 (%)

Comparator – central slot accommodates hemoglobin tube and on either side non-fading brown tinted glass pieces for colour matching are present

Stirrer – thin glass rod for stirring the solution


Sahlis hemoglobinometre

N/10 HCl-To make 500 ml of N/10 HCl mix

Concentrated HCl – 4.5ml

Distilled water – 500ml Distilled water

Procedure

Add N/10 HCl to the hemoglobinometer tube up to its lowest mark i.e 2g% (If N/10 HCl is taken above the mark the color of undiluted solution is lighter than standard and if N/10 HCl is taken less then all the hemoglobin is not converted).

Take blood up to 20 cu mm mark on the pipette and transfer it to the hemoglobinometer tube containing N/10 HCl.

Leave the solution for 10 minutes (for the conversion of hemoglobin to acid hematin)

After 10 minutes add distilled water drop by drop and mix it by stirrer until the color matches with the color of comparator. While matching the color glass rod should be removed from the solution.

The lower meniscus of the solution should be taken as the result which expresses hemoglobin content as g%

If the hemoglobin is too low (less than 3g/dl) then 40µl blood is added to HCl upto 20 marks in the tube. Color is matched and result is halved

Advantages

Inexpensive

Easy to perform

Requires no technical skill

Can be used as bed side procedure

Reagents and apparatus are free

Disadvantages

As Acid hematin is unstable, color fades away quickly

Visual error is possible while matching with brown color of comparator box

Technical errors like improper mixing of blood, errors in pipetting and capillary blood with tissue fluid can give false results

No international standard for brown coloured comparator box

If the match point is passed then the whole process has to be repeated

Not all types of hemoglobin are converted to acid hematin (eg.sulfhemoglobin, methemoglobin)

SPECTROPHOTOMETRIC METHODS

CYANMETHEMOGLOBIN METHOD

This is the most accurate method.

Principle – when the blood is mixed with a solution containing potassium ferricyanide and potassium cyanide, all types of hemoglobins except sulfhemoglobin is converted to cyanmethemoglobin. The intensity of color is

proportional to hemoglobin concentration and it is compared with a known cyanmethemoglobin standard at 540nm (green filter) in a photoelectric colorimeter.

Reagents used

Drabkins reagent

Distilled water – 1000ml

Potassium ferricyanide – 200mg

Potassium dihydrogen phosphate – 140mg

Potassium cyanide – 50mg

Non-ionic detergent – 1ml

pH of Drabkins solution is 7.0 to 7.4

Drabkins reagent is clear and pale yellow.

It should be stored in brown borosilicate bottles as it is unstable if exposed to light.

If temperature is high should be refrigerated at 2 to 80 (but never freeze) and should be brought to room temperature before using it.

When measured in a spectrophotometer against water as a blank at the wavelength of 540nm, the absorbance must be zero

Drabkins solution should not be used if

pH is outside the range

fluid is turbid

its absorbance in spectrophotometer is not zero at 540nm

Cyanmethemoglobin standard solution

commercially available

Standard is directly pipetted in a cuvette and optical density measured at 540nm (green filter). Reading obtained corresponds to 15g/dl of hemoglobin for dilution of 1:250

Necessary to store this hemoglobin standard at 2 – 80C

Bring this standard solution to room temperature before recording its optical density on photometer.

Procedure

20µl of blood is added to 5ml of Drabkins reagent and mixed well. Leave it for 5 minutes. Now measure the absorbance of this solution and standard in spectrophotometer at 540nm after adjusting the OD at 0 by using Drabkins solution as blank

Calculation – Hbg g/dl – 15 X OD test / OD standard

Advantages

Visual error is absent as there is no color matching

Cyanmethemoglobin is stable so it does not fade away quickly

All forms of hemoglobin except Sulfhemoglobin are converted to cyanmethemoglobin

Reliable standard reference is available from WHO for direct comparision

Disadvantages

Potassium cyanide is poisonous and should not be pipetted by mouth

Aftr dilution solution should be kept for some time for complete conversion

Rate of conversion of blood containing carboxy hemoglobin is long (30 minutes)

Blood with abnormal plasma proteins and high leukocyte count may cause turbidity on dilution of blood

OXYHEMOGLOBIN METHOD

0.007N Ammonium hydroxide is added to blood which causes hemolysis of red cells and converts hemoglobin to oxyhemoglobin. The color of the solution is measured in the spectrophotometer at wave length of 540nm.

Advantage – simple , quick and reliability is not affected by increased bilirubin level

Disadvantages –Oxyhemoglobin solution fades quickly.

Method does not give satisfactory results in the presence of methemoglobin, sulfhemoglobin and carboxyhemoglobin.

Stable standard solution cannot be prepared

CONDITIONS WITH VARIATIONS IN HAEMOGLOBIN LEVEL

Conditions where hemoglobin are raised

Chronic obstructive pulmonary disease

Congenital cyanotic disease of heart

Smokers polycythemia

Renal cell carcinoma – due to ectopic secretion of erythropoietin

Pheochromocytoma

Polycythemia vera

People living in high altitude

Conditions where hemoglobin is falsely raised

Burns

Severe dehydration

Immediately after acute hemorrhage

Blood taken during the intravenous infusion of iron containing drugs

Conditions with false anemia

Pregnancy – due to increase in plasma volume leading to fall of 1 to 2 g/dl

Hypervolemia – due to disproportionate increase in plasma volume and RBC volume

Causes for decreased hemoglobin concentration

Anemia due to

Blood loss

Parasitic infection

Drugs and lead poisoning

Dietary deficiency (iron, copper, vitamins)

Malabsorption of nutrients

Chronic disease (diseases of liver, kidney and cancer)

Physiological variations

Strenuous physical exercise

Diurnal variation – highest in morning and lowest in evening

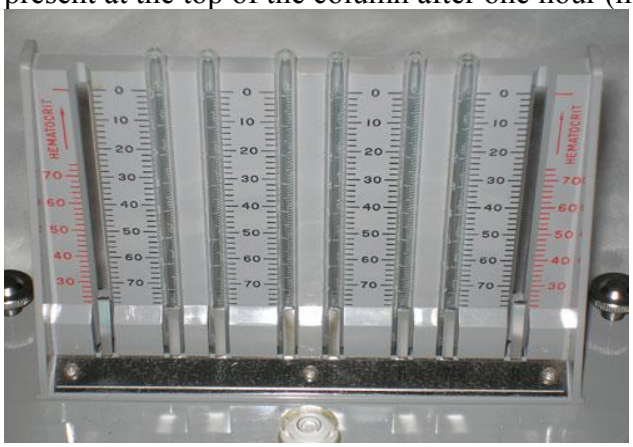
High altitude – increase with increase in altitude

Erythrocyte sedimentation rate (ESR)

The ESR is a simple non-specific screening test that indirectly measures the presence of inflammation in the body. It reflects the tendency of red blood cells to settle more rapidly in the face of some disease states, usually because of increases in plasma fibrinogen, immunoglobulins, and other acute-phase reaction proteins. Changes in red cell shape or numbers may also affect the ESR.

Method:

When anticoagulated whole blood is allowed to stand in a narrow vertical tube for a period of time, the RBCs – under the influence of gravity - settle out from the plasma. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr)



The Wintrobe sedimentation rack

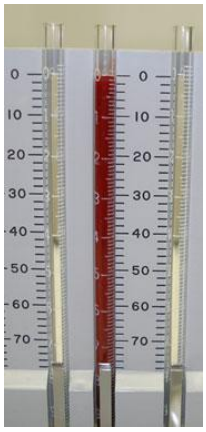
There are two main methods used to measure the ESR: the Westergren method and the Wintrobe Method. Each method produces slightly different results. Most laboratories use the Westergren method.

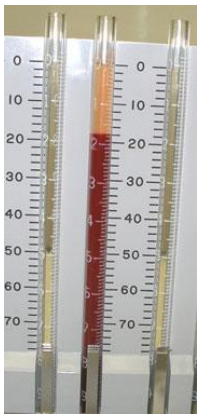
Westergren method:

The Westergren method requires collecting 2 ml of venous blood into a tube containing 0 .5 ml of sodium citrate. It should be stored no longer than 2 hours at room temperature or 6 hours at 4 °C. The blood is drawn into a Westergren-Katz tube to the 200 mm mark. The tube is placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The distance of fall of erythrocytes, expressed as millimeters in 1 hour, is the ESR.

Wintrobe method:

The Wintrobe method is performed similarly except that the Wintrobe tube is smaller in diameter than the Westergren tube and only 100 mm long. EDTA anticoagulated blood without extra diluent is drawn into the tube, and the rate of fall of red blood cells is measured in millimeters after 1 hour. The shorter column makes this method less sensitive than the Westergren method because the maximal possible abnormal value is lower. However, this method is more practical for demonstration purposes.





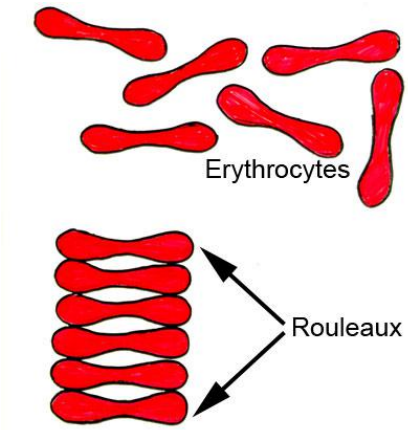
2.Average values in healthy men are: <15mm/hr; in healthy females, they are somewhat higher: <20mm. The values are slightly higher in old age, in both genders.

1.This picture shows a rack holding Wintrobe tubes, in which anticoagulated whole blood has just been added.(Time: 0)

Theoretical considerations

The RBCs sediment because their density is greater than that of plasma; this is particularly so, when there is an alteration in the distribution of charges on the surface of the RBC (which normally keeps them separate form each other) resulting in their coming together to form large aggregates known as rouleaux.

Rouleaux formation is determined largely by increased levels of plasma fibrinogen and globulins, and so the ESR reflects mainly changes in the plasma proteins that accompany acute and chronic infections, some tumors and degenerative diseases. In such situations, the ESR values are much greater than 20mm/hr. Note that the ESR denotes merely the presence of tissue damage or disease, but not its severity; it may be used to follow the progress of the diseased state, or monitor the effectiveness of treatment



Thalassemia

Thalassemia is an inherited blood disorder in which the body makes an abnormal form of hemoglobin. Hemoglobin is the protein molecule in red blood cells that carries oxygen.

The disorder results in excessive destruction of red blood cells, which leads to anemia. Anemia is a condition in which your body doesn't have enough normal, healthy red blood cells.

Thalassemia is inherited, meaning that at least one of your parents must be a carrier of the disorder. It's caused by either a genetic mutation or a deletion of certain key gene fragments.

Thalassemia minor is a less serious form of the disorder. There are two main forms of thalassemia that are more serious. In alpha thalassemia, at least one of the alpha globin genes has a mutation or abnormality. In beta thalassemia, the beta globin genes are affected.

Each of these forms of thalassemia has different subtypes. The exact form you have will affect the severity of your symptoms and your outlook.

Symptoms of thalassemia

The symptoms of thalassemia can vary. Some of the most common ones include:

- bone deformities, especially in the face
- dark urine
- delayed growth and development
- excessive tiredness and fatigue
- yellow or pale skin

Not everyone has visible symptoms of thalassemia. Signs of the disorder also tend to show up later in childhood or adolescence.

Causes of thalassemia

Thalassemia occurs when there's an abnormality or mutation in one of the genes involved in hemoglobin production. You inherit this genetic abnormality from your parents.

If only one of your parents is a carrier for thalassemia, you may develop a form of the disease known as thalassemia minor. If this occurs, you probably won't have symptoms, but you'll be a carrier. Some people with thalassemia minor do develop minor symptoms.

If both of your parents are carriers of thalassemia, you have a greater chance of inheriting a more serious form of the disease.

Thalassemia is most commonTrusted Source in people from Asia, the Middle East, Africa, and Mediterranean countries such as Greece and Turkey.

Different types of thalassemia

There are three main types of thalassemia (and four subtypes):

- beta thalassemia, which includes the subtypes major and intermedia
- alpha thalassemia, which include the subtypes hemoglobin H and hydrops fetalis
- thalassemia minor

All of these types and subtypes vary in symptoms and severity. The onset may also vary slightly.

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Diagnosis thalassemia

If your doctor is trying to diagnose thalassemia, they'll likely take a blood sample. They'll send this sample to a lab to be tested for anemia and abnormal hemoglobin. A lab technician will also look at the blood under a microscope to see if the red blood cells are oddly shaped.

Abnormally shaped red blood cells are a sign of thalassemia. The lab technician may also perform a test known as hemoglobin electrophoresis. This test separates out the different molecules in the red blood cells, allowing them to identify the abnormal type.

Depending on the type and severity of the thalassemia, a physical examination might also help your doctor make a diagnosis. For example, a severely enlarged spleen might suggest to your doctor that you have hemoglobin H disease.

Treatment options for thalassemia

The treatment for thalassemia depends on the type and severity of disease involved. Your doctor will give you a course of treatment that will work best for your particular case.

Some of the treatments include:

- blood transfusions
- bone marrow transplant
- medications and supplements
- possible surgery to remove the spleen or gallbladder

Your doctor may instruct you not to take vitamins or supplements containing iron. This is especially true if you need blood transfusions because people who receive them accumulate extra iron that the body can't easily get rid of. Iron can build up in tissues, which can be potentially fatal.

If you're receiving a blood transfusion, you may also need chelation therapy. This generally involves receiving an injection of a chemical that binds with iron and other heavy metals. This helps remove extra iron from your body.

Thalassemia beta

Beta thalassemia occurs when your body can't produce beta globin. Two genes, one from each parent, are inherited to make beta globin. This type of thalassemia comes in two serious subtypes: thalassemia major (Cooley's anemia) and thalassemia intermedia.

Thalassemia major

Thalassemia major is the most severe form of beta thalassemia. It develops when beta globin genes are missing.

The symptoms of thalassemia major generally appear before a child's second birthday. The severe anemia related to this condition can be life-threatening. Other signs and symptoms include:

- fussiness
- paleness
- frequent infections
- a poor appetite
- failure to thrive
- jaundice, which is a yellowing of the skin or the whites of the eyes
- enlarged organs

This form of thalassemia is usually so severe that it requires regular blood transfusions.

Thalassemia intermedia

Thalassemia intermedia is a less severe form. It develops because of alterations in both beta globin genes. People with thalassemia intermedia don't need blood transfusions.

Thalassemia alpha

Alpha thalassemia occurs when the body can't make alpha globin. In order to make alpha globin, you need to have four genes, two from each parent.

This type of thalassemia also has two serious types: hemoglobin H disease and hydrops fetalis.

Hemoglobin H

Hemoglobin H develops as when a person is missing three alpha globin genes or experiences changes in these genes. This disease can lead to bone issues. The cheeks, forehead, and jaw may all overgrow. Additionally, hemoglobin H disease can cause:

- jaundice
- an extremely enlarged spleen
- malnourishment

Hydrops fetalis

Hydrops fetalis is an extremely severe form of thalassemia that occurs before birth. Most babies with this condition are either stillborn or die shortly after being born. This condition develops when all four alpha globin genes are altered or missing.

Thalassemia and anemia

Thalassemia can quickly lead to anemia. This condition is marked by a lack of oxygen being transported to tissues and organs. Since red blood cells are responsible for delivering oxygen, a reduced number of these cells means you don't have enough oxygen in the body either.

Your anemia may be mild to severe. Symptoms of anemia include:

- dizziness
- fatigue
- irritability
- shortness of breath
- weakness

Anemia can also cause you to pass out. Severe cases can lead to widespread organ damage, which can be fatal.

Thalassemia and genetics

Thalassemia is genetic in nature. To develop full thalassemia, *both* of your parents must be carriers of the disease. As a result, you will have two mutated genes.

It's also possible to become a carrier of thalassemia, where you only have one mutated gene and not two from both parents. Either one or both of your parents must have the condition or be a *carrier* of it. This means that you inherit one mutated gene from either one of your parents.

It's important to get tested if one of your parents or a relative has some form of the disease.

Thalassemia minor

In alpha minor cases, two genes are missing. In beta minor, one gene is missing. People with thalassemia minor don't usually have any symptoms. If they do, it's likely to be minor anemia. The condition is classified as either alpha or beta thalassemia minor.

Even if thalassemia minor doesn't cause any noticeable symptoms, you can still be a carrier for the disease. This means that, if you have children, they could develop some form of the gene mutation.

Thalassemia in children

Of all the babies born with thalassemia each year, it's estimated that 100,000 are born with severe forms worldwide.

Children can start exhibiting symptoms of thalassemia during their first two years of life. Some of the most noticeable signs include:

- fatigue
- jaundice
- pale skin
- poor appetite
- slow growth

It's important to diagnose thalassemia quickly in children. If you or your child's other parent are carriers, you should have testing done early.

When left untreated, this condition can lead to problems in the liver, heart, and spleen. Infections and heart failure are the most common life-threatening complications of thalassemia in children.

Like adults, children with severe thalassemia need frequent blood transfusions to get rid of excess iron in the body.

Diet for thalassemia

A low-fat, plant-based diet is the best choice for most people, including those with thalassemia. However, you may need to limit iron-rich foods if you already have high iron levels in your blood. Fish and meats are rich in iron, so you may need to limit these in your diet.

You may also consider avoiding fortified cereals, breads, and juices. They contain high iron levels, too.

Thalassemia can cause folic acid (folate) deficiencies. Naturally found in foods such as dark leafy greens and legumes, this B vitamin is essential for warding off the effects of high iron levels and protecting red blood cells. If you're not getting enough folic acid in your diet, your doctor may recommend a 1 mg supplement taken daily.

There's no one diet that can cure thalassemia, but making sure you eat the right foods can help. Be sure to discuss any dietary changes with your doctor ahead of time.

Prognosis

Since thalassemia is a genetic disorder, there's no way to prevent it. However, there are ways you can manage the disease to help prevent complications.

In addition to ongoing medical care, the CDC recommendsTrusted Source that all individuals with disorder protect themselves from infections by keeping up with the following vaccines:

- haemophilus influenza type b
- hepatitis
- meningococcal
- pneumococcal

In addition to a healthy diet, regular exercise can help manage your symptoms and lead to a more positive prognosis. Moderate-intensity workouts are usually recommended, since heavy exercise can make your symptoms worse.

Walking and bike riding are examples of moderate-intensity workouts. Swimming and yoga are other options, and they're also good for your joints. The key is to find something you enjoy and keep moving.

Life expectancy

Thalassemia is a serious illness that can lead to life-threatening complications when left untreated or undertreated. While it's difficult to pinpoint an exact life expectancy, the general rule is that the more severe the condition, the quicker thalassemia can become fatal.

According to some estimates, people with beta thalassemia — the most severe form — typically die by age 30. The shortened life span has to do with iron overload, which can eventually affect your organs.

Researchers are continuing to explore genetic testing as well as the possibility of gene therapy. The earlier thalassemia is detected, the sooner you can receive treatment. In the future, gene therapy could possibly reactivate hemoglobin and deactivate abnormal gene mutations in the body.

How does thalassemia affect pregnancy?

Thalassemia also brings up different concerns related to pregnancy. The disorder affects reproductive organ development. Because of this, women with thalassemia may encounter fertility difficulties.

To ensure the health of both you and your baby, it's important to plan ahead of time as much as possible. If you want to have a baby, discuss this with your doctor to make sure that you're in the best health possible.

Your iron levels will need to be carefully monitored. Preexisting issues with major organs are also considered.

Prenatal testing for thalassemia may be done at 11 and 16 weeks. This is done by taking fluid samples from either the placenta or the fetus, respectively.

Pregnancy carries the following risk factors in women with thalassemia:

- a higher risk for infections
- gestational diabetes
- heart problems
- hypothyroidism, or low thyroid
- increased number of blood transfusions
- low bone density

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UNIT – III - DISORDERS OF METABOLISM-I I & II SBC3104

UNIT 3 DISORDERS OF METABOLISM-I

Diseases related to carbohydrate metabolism: Diabetes mellitus:- Types, Clinical features, complications, GTT, galactosaemia, fructosuria, and glycogen storage diseases. Disorders in lipid metabolism: Atherosclerosis – aetiology, clinical features and its complications. Lipid storage diseases and fatty liver.

DISORDERS OF METABOLISM-II

Disorders in protein metabolism: Phenylketonuria, alkaptonuria, cystinuria, albinism and tyrosinemia. Disorders in nucleic acid metabolism: Gout:- Types, aetiology and clinical features. Disorders in bilirubin metabolism: Jaundice:- classification, clinical features.

UNIT -III

DISORDERS OF METABOLISM - I & II

Disorders Of Carbohydrate Metabolism

The metabolism of the carbohydrates galactose, fructose, and glucose is intricately linked through interactions between different enzymatic pathways, and disorders that affect these pathways may have symptoms ranging from mild to severe or even life-threatening. Clinical features include various combinations of hypoglycemia (low blood sugar), liver enlargement, and muscle pain. Most of these disorders can be treated, or at least controlled, with specific dietary interventions.

Galactose and fructose disorders

Galactosemia usually is caused by a defective component of the second major step in the metabolism of the sugar galactose. When galactose is ingested, as in milk, galactose-1-phosphate accumulates. Therefore, the clinical manifestations of galactosemia begin when milk feeding is started. If the feeding is not stopped, infants with the disorder will develop lethargy, jaundice, progressive liver dysfunction, kidney disease, and weight loss. They are also susceptible to severe bacterial infections, especially by *Escherichia coli*. Cataracts develop if the diet remains galactose-rich. Intellectual disability occurs in most infants with galactosemia if the disorder is left untreated or if treatment is delayed. Therapy is by exclusion of galactose from the diet and results in the reversal of most symptoms. Most children have normal intelligence, although they may have learning difficulties and a degree of intellectual disability despite early therapy.

Hereditary fructose intolerance (HFI) is caused by a deficiency of the liver enzyme fructose-1-phosphate aldolase. Symptoms of HFI appear after the ingestion of fructose and thus present later in life than do those of galactosemia. Fructose is present in fruits, table sugar (sucrose), and infant formulas containing sucrose. Symptoms may include failure to gain weight satisfactorily, vomiting, hypoglycemia, liver dysfunction, and kidney defects. Older children with HFI tend to avoid sweet foods and may have teeth notable for the absence of caries. Children with the disorder do very well if they avoid dietary fructose and sucrose.

Fructose 1,6-diphosphatase deficiency is associated with an impaired ability to form glucose from other substrates (a process called gluconeogenesis). Symptoms include severe hypoglycemia, intolerance to fasting, and enlargement of the liver. Rapid treatment of hypoglycemic episodes with intravenous fluids containing glucose and the avoidance of fasting are the mainstays of therapy. Some patients require continuous overnight drip feeds or a bedtime dose of cornstarch in order to control their tendency to develop hypoglycemia.

Glycogen storage disorders

The brain, red blood cells, and inner portion of the adrenal gland (adrenal medulla) depend on a constant supply of glucose for their metabolic functions. This supply begins in the small intestine, where transport proteins mediate the uptake of glucose into cells lining the gut. Glucose subsequently passes into the bloodstream and then the liver, where it is stored as glycogen. In times of starvation or fasting or when the body requires a sudden energy supply, glycogen is broken down into glucose, which is then released into the blood. Muscle tissue also has its own glycogen stores, which may be degraded during exercise. If enzymes responsible for glycogen degradation are blocked so that glycogen remains in the liver or muscle, a number of conditions known as glycogen storage disorders (GSD) can arise. Depending upon which enzyme is affected, these conditions may affect the liver, muscles, or both. In GSD type I (von Gierke disease), the last step in glucose release from the liver is defective, leading to hypoglycemia. Therapy consists of supplying continuous glucose to the digestive tract (e.g., by continuous drip feedings) during infancy and early childhood. As the child grows, an improvement in symptoms tends to occur. Adequate glucose is supplied by frequent feedings of carbohydrates and slow-release glucose (uncooked cornstarch) before bedtime. Liver transplantation may also be curative, but this drastic measure is reserved for the small percentage of patients who do not respond to the usual treatment or who develop liver cancer. For the muscular forms of the disease, avoidance of strenuous exercise is the usual therapy. Defects in earlier steps in glycogen breakdown in the liver cause GSD types III, IV, VI, and IX, which usually lead to milder versions of type I disease. Pompe disease (GSD type II) is discussed in the section Lysosomal storage disorders.

In addition to glycogen degradation, glucose may be manufactured from amino acids and pyruvate in the process of gluconeogenesis. Key enzymes in the gluconeogenic pathway include carboxylase, phosphoenolpyruvate carboxykinase, and fructose-1,6-diphosphatase. Persons with defects in these enzymes develop conditions including fasting hypoglycemia, lactic acidemia, and liver enlargement. Thus, gluconeogenesis disorders may be difficult to distinguish from glycogen storage disorders at first presentation.

Congenital disorders of glycosylation

Congenital disorders of glycosylation (CDG; formerly known as carbohydrate-deficient glycoprotein syndrome) are recently described diseases that

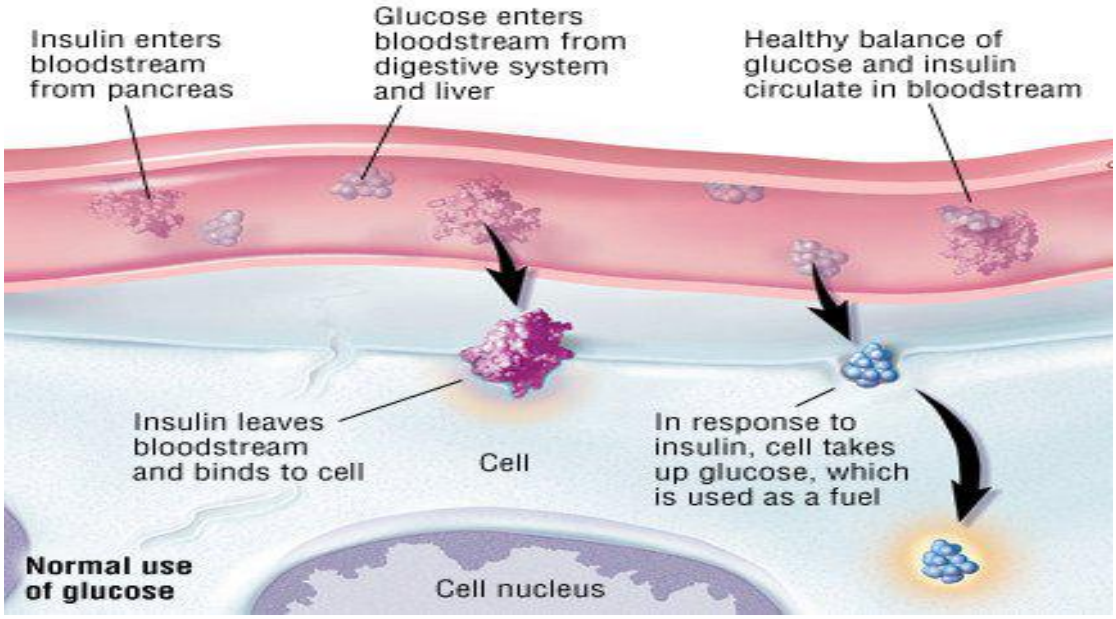
affect the brain and many other organs. The primary biochemical defects of CDG are in the N-glycosylation pathway that occurs in the cytoplasm and endoplasmic reticulum, cellular organelles involved in the synthesis of proteins and lipids. A defect in a mannose-processing enzyme, phosphomannomutase 2, causes the most common form of CDG (type I). Other enzymatic defects have been identified, but the biochemical bases of some CDG subtypes have not yet been determined. The classic form of CDG (type Ia) is characterized by low muscle tone in infancy, severe developmental delay, and brain abnormalities. Children with type Ia also have inverted nipples and an unusual distribution of fat, especially in the suprapubic region and buttocks. Other features include hypoglycemia, seizures, stroke-like episodes, retinal damage, impaired heart contractility, vomiting, liver disease, diarrhea, and a bleeding tendency. No effective therapy exists for CDG, except for the rare type Ib disease (phosphomannose isomerase deficiency), in which oral administration of mannose may reverse symptoms in some cases.

DIABETES MELLITUS :- Types, Clinical features, complications

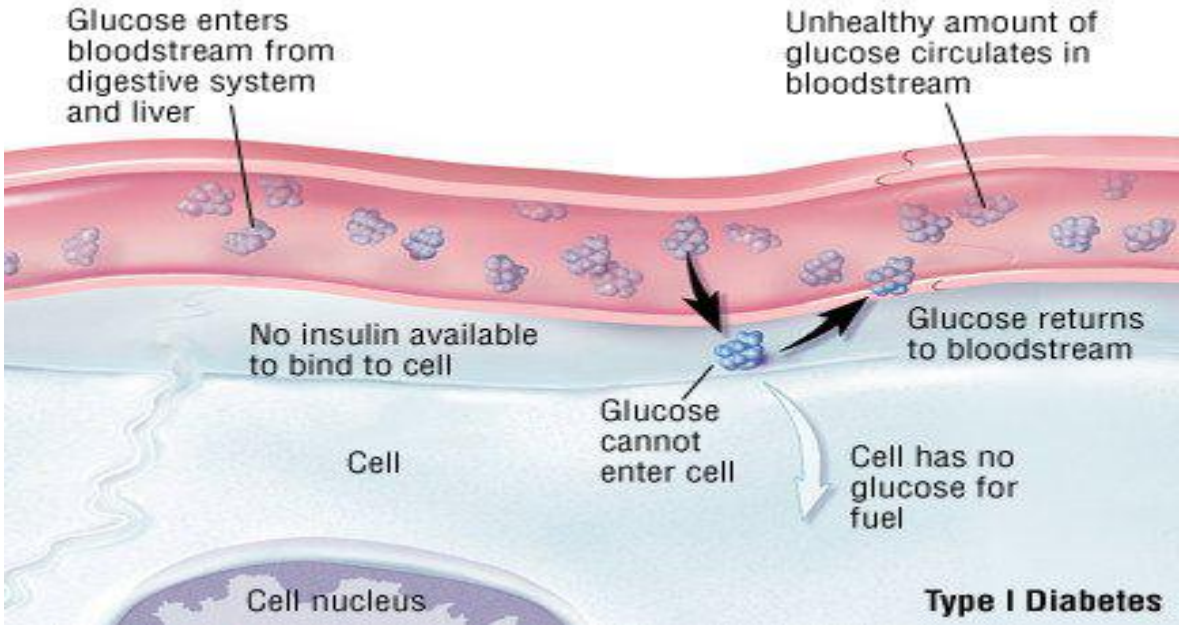
Diabetes mellitus is a condition defined by persistently high levels of sugar (glucose) in the blood. There are several types of diabetes. The two most common are called type 1 diabetes and type 2 diabetes.

During digestion, food is broken down into its basic components. Carbohydrates are broken down into simple sugars, primarily glucose. Glucose is a critically important source of energy for the body’s cells. To provide energy to the cells, glucose needs to leave the bloodstream and get inside the cells.

An organ in the abdomen called the pancreas produces a hormone called **insulin**, which is essential to helping glucose get into the body's cells. In a person without diabetes, the pancreas produces more insulin whenever blood levels of glucose rise (for example, after a meal), and the insulin signals the body's cells to take in the glucose. In diabetes, either the pancreas's ability to produce insulin or the cells' response to insulin is altered.



Type 1 diabetes is an autoimmune disease. This means it begins when the body's immune system mistakenly attacks other cells in the body. In type 1 diabetes, the immune system destroys the insulin-producing cells (called beta cells) in the pancreas. This leaves the person with little or no insulin in his or her body. Without insulin, glucose accumulates in the bloodstream rather than entering the cells. As a result, the body cannot use this glucose for energy. In addition, the high levels of blood glucose cause excessive urination and dehydration, and damage the body's tissues.



Type 2 diabetes occurs when your body's cells become less responsive to insulin's efforts to drive glucose into the cells, a condition called insulin resistance. As a result, glucose starts to build up in the blood.

In people with insulin resistance, the pancreas "sees" the blood glucose level rising. The pancreas responds by making extra insulin to try to usher the glucose into the cells. At first, this works, but over time, the body's insulin resistance gets worse. In response, the pancreas makes more and more insulin. Finally, the pancreas gets "exhausted." It cannot keep up with the demand for more and more insulin. As a result, blood glucose levels rise and stay high.

Type 2 diabetes is also called adult-onset diabetes. That's because it almost always used to start in middle or late adulthood. However, more and more children and teens are now developing this condition.

Type 2 diabetes is much more common than type 1 diabetes. It tends to run in families. Obesity also increases your risk of type 2 diabetes. It is truly a different disease than type 1 diabetes, although both types involve a high blood glucose level and the risk of complications associated with it.

Another kind of diabetes, called gestational diabetes, happens in women who have higher-than-expected blood sugar levels during pregnancy. Once it occurs, it lasts throughout the remainder of the pregnancy. Like the other types of diabetes, gestational diabetes happens when the hormone insulin can't efficiently move sugar (glucose) into the body's cells so it can be used as fuel. In gestational diabetes, the body does not respond well to insulin, unless insulin can be produced or provided in larger amounts.

In most women, the disorder goes away when the pregnancy ends, but women who have had gestational diabetes are at increased risk of developing type 2 diabetes later.

Symptoms

Diabetes initially might not cause any symptoms. It can sometimes be caught early with a routine blood test before a person develops symptoms.

When diabetes does cause symptoms, they may include:

- excessive urination
- excessive thirst, leading to drinking a lot of fluid
- weight loss.

People with diabetes also have an increased susceptibility to infections, especially yeast (Candida) infections.

When the amount of insulin in the blood stream is too low, extremely high blood sugar levels can lead to dangerous complications. The body can become too acidic, a

condition called diabetic ketoacidosis. Or the blood sugar level gets so high, the person becomes severely dehydrated. It's called hyperosmolar syndrome.

The symptoms of these complications include confused thinking, weakness, nausea, vomiting, and even seizures and coma. In some cases, diabetic ketoacidosis or hyperosmolar syndrome is the first sign that a person has diabetes.

The treatment of diabetes also can produce symptoms. Too much glucose-lowering medicine, relative to dietary intake, can lead to a blood sugar level that has dropped too low (called hypoglycemia). Symptoms of hypoglycemia include:

- sweating
- trembling
- dizziness
- hunger
- confusion
- seizures and loss of consciousness (if hypoglycemia is not recognized and corrected).

You can correct hypoglycemia by eating or drinking something that has carbohydrates. This raises your blood sugar level.

Long-term diabetes can have other complications, including:

- **Atherosclerosis** — Atherosclerosis is fat buildup in the artery walls. This can impair blood flow to all parts of the body. The heart, brain, and legs are affected most often.
- **Retinopathy** — Tiny blood vessels in the retina (the part of the eye that sees light) can become damaged by high blood sugar. The damage can block blood flow to the retina, or can lead to bleeding into the retina. Both reduce the retina's ability to see light. Caught early, retinopathy damage can be minimized by tightly controlling blood sugar and using laser therapy. Untreated retinopathy can lead to blindness.
- **Neuropathy** — This is another term for nerve damage. The most common type is peripheral neuropathy, which affects nerves in the feet and hands. The nerves to the legs are damaged first, causing pain and numbness in the feet. This can advance to cause symptoms in the legs and hands. Damage to the nerves that control digestion, sexual function, and urination can also occur.
- **Foot problems** — Any sores, injuries, or blisters on the feet can lead to the following complications:
  - If peripheral neuropathy causes numbness, a person may not feel any irritation or injury that occurs on the foot. The skin can break down and form an ulcer, and the ulcer can get infected.

- Blood circulation can be poor, leading to slow healing of any foot injuries. Left untreated, a simple sore can become very large and get infected. If medical treatment cannot heal the sore, an amputation may be required.

- **Nephropathy** — This refers to damage to the kidneys. This complication is more likely if blood sugars remain elevated and high blood pressure is not treated aggressively.

Diagnosis

Diabetes is diagnosed through blood tests that detect the level of glucose in the blood.

- **Fasting plasma glucose (FPG) test.** A blood sample is taken in the morning after you fast overnight. A normal fasting blood sugar level is between 70 and 100 milligrams per deciliter (mg/dL). Diabetes is diagnosed if the fasting blood sugar level is 126 mg/dL or higher.

- **Oral glucose tolerance test (OGTT).** Your blood sugar is measured two hours after you drink a liquid containing 75 grams of glucose. Diabetes is diagnosed if the blood sugar level is 200 mg/dL or higher.

- **Random blood glucose test.** A blood sugar of 200 mg/dL or greater at any time of day, combined with symptoms of diabetes, is sufficient to make the diagnosis.

- **Hemoglobin A1c (glycohemoglobin).** This test measures your average blood glucose level over the prior two to three months. Diabetes is diagnosed if the hemoglobin A1c level is 6.5% or higher.

Expected Duration

Type 1 diabetes is a lifelong illness. Usually, type 2 diabetes is also life-long. However, people with type 2 diabetes can sometimes restore their blood sugar levels to normal just by eating a healthy diet, exercising regularly, and losing weight.

Gestational diabetes usually goes away after childbirth. However, women with gestational diabetes are at high risk for developing type 2 diabetes later in life.

In people with diabetes, aging and episodic illnesses can cause the body's insulin resistance to increase. As a result, additional treatment typically is required over time.

Prevention

Type 1 diabetes cannot be prevented.

You can decrease your risk of developing type 2 diabetes.

If a close relative—particularly, a parent or sibling—has type 2 diabetes, or if your blood glucose test shows "pre-diabetes" (defined as blood glucose levels between 100 and 125 mg/dL), you are at increased risk for developing type 2 diabetes. You can help to prevent type 2 diabetes by

- maintaining your ideal body weight.
- exercising regularly—such as a brisk walk of 1-2 miles in 30 minutes—at least five times a week, even if that does not result in you achieving an ideal weight. That's because regular exercise reduces insulin resistance even if you don't lose weight.
- eating a healthy diet.
- taking medication. The medication **metformin** (**Glucophage**) offers some additional protection for people with pre-diabetes.

If you already have type 2 diabetes, you can still delay or prevent complications by doing the following.

Keep control of your blood sugar. This helps reduce the risk of most complications.

Lower your risk of heart-related complications. Aggressively manage other risk factors for atherosclerosis, such as:

- high blood pressure
- high cholesterol and triglycerides
- cigarette smoking
- obesity

Visit an eye doctor and a foot specialist every year. This can help you reduce the risk of eye and foot complications.

Treatment

Type 1 diabetes is always treated with insulin injections.

In most cases, type 2 diabetes treatment begins with weight reduction through diet and exercise. A healthy diet for a person with diabetes is low in total calories, free of trans fats and nutritionally balanced, with abundant amounts of whole grains, fruits and vegetables, and monounsaturated fats.

Most people with type 2 diabetes need drug therapy to control blood sugar. However, it is possible to achieve normal blood sugar levels with weight loss, a healthy diet and regular exercise.

Even if medications are required, diet and exercise remain important for controlling diabetes.

The medications used for type 2 diabetes include pills and injections. They work in many different ways. They include medications that:

- reduce insulin resistance in the muscles and liver
- increase the amount of insulin made and released by the pancreas
- provide additional insulin
- cause a burst of insulin release with each meal
- delay the absorption of sugars from the intestine
- slow your digestion
- reduce your appetite for large meals
- decrease the conversion of fat to glucose.

Weight loss surgery may be an option for some obese people with type 2 diabetes.

When To Call a Professional

If you have diabetes, see your doctor regularly.

People with high blood sugar levels have a higher risk of dehydration. Contact your doctor immediately if you develop vomiting or diarrhea and are not able to drink enough fluids.

Monitor your blood sugar as advised by your health care team. Report any significant deviations in blood sugar levels.

Prognosis

The prognosis in people with diabetes varies. It depends on how well an individual modifies his or her risk of complications. If blood sugar is not well controlled, it can increase a person's risk of heart attack, stroke, and kidney disease, which can result in premature death. Disability due to blindness, amputation, heart disease, stroke, and nerve damage may occur. Some people with diabetes become dependent on dialysis treatments because of kidney failure.

Fructosuria, disturbance of fructose metabolism resulting from a hereditary disorder or intolerance. Normally, fructose is first metabolized in the body to fructose-1-phosphate by a specific organic catalyst or enzyme called fructokinase. In fructosuria this particular enzyme is defective, and the concentration of fructose increases in the blood and urine. There are no other clinical manifestations or disabilities, and the condition is compatible with normal life expectancy.

Disorders of Lipid Metabolism

The initial evaluation consists of a history and physical examination, including assessment of CHD risk factors and measurement of plasma lipids. Exclusion of secondary causes of lipid disorders is important. Obesity is an independent risk factor for CHD not included as a traditional risk factor, although it is reflected in the waist circumference measurement that is used to define the metabolic syndrome. Obesity aggravates dyslipidemia, hypertension, and insulin resistance and is a target of therapy regardless of the severity of traditional CHD risk factors. Particular emphasis should be placed on obtaining a detailed history of all first-degree relatives to identify cholesterol disorders or premature CHD.

Physical Examination

The examination should emphasize the cardiovascular system, manifestations of hyperlipidemia, and disorders causing secondary lipid abnormalities. Several unique clinical findings

Xanthelasmas a type of xanthoma, are raised, yellowish macules that typically appear around the medial canthus. Involvement can extend to the eyelids or skin immediately below the eye. They occur in patients with FH, familial defective apoB100, or dysbetalipoproteinemia. They occasionally occur in patients with normal cholesterol levels. Xanthelasmas typically regress with cholesterol lowering and may be treated effectively in the setting of normal cholesterol levels with cholesterol-lowering drugs.

Lipemia retinalis a condition in which lipemic blood causes opalescence of retinal arterioles, can be observed during funduscopic examination. It is typically seen only when the triglyceride levels are 22.6 mmol/L (2000 mg/dL) or higher.

Tendon xanthomas are nodular deposits of cholesterol that accumulate in tissue macrophages in the Achilles and other tendons, including the extensor tendons in the hands, knees, and elbows. Tendon xanthomas are often present in patients with FH or familial defective apoB100 and sometimes in those with dysbetalipoproteinemia. As discussed earlier, the Achilles tendon should be palpated for assessment of thickness and contour.

Tuberous or tuboeruptive xanthomas develop in areas that are susceptible to trauma, such as the elbows and knees. They range from pea-sized to lemon-sized and can be seen in dysbetalipoproteinemia and FH. Palmar xanthomas are found in the palmar and digital creases of the hands. This type of xanthoma is almost pathognomonic for high plasma levels of β-VLDL and dysbetalipoproteinemia. Eruptive xanthomas appear as small, yellowish, round papules that contain a pale center and an erythematous base. Their distribution includes the abdominal wall, back, buttocks, and other pressure contact areas. They are caused by accumulation of triglyceride in dermal histiocytes and typically occur when the plasma triglyceride level is 11.3 to 22.6 mmol/L (1000 to 2000 mg/dL) or higher. They can disappear rapidly with lowering of the plasma triglyceride concentration.

Screening for Secondary Disorders

The history and physical examination should be directed toward uncovering secondary disorders of lipid metabolism and identifying agents including medications that could cause hyperlipidemia. Minimal studies should include fasting blood glucose, glycosylated hemoglobin, renal and hepatic function tests, urinary protein, and thyroid-stimulating hormone.

Measurement of Plasma Lipids

Ideally, plasma lipids should be measured at least twice under fasting steady-state conditions before therapeutic decisions are made. Plasma lipids are usually measured after a 12-hour fast due to postprandial fluctuations of triglycerides. Because cholesterol is a minor component of chylomicrons, total plasma cholesterol can be measured in either a fasting or a nonfasting state. Plasma lipid measurements are usually reliable if done within the first 24 hours after an acute myocardial infarction.

Most clinical laboratories measure plasma levels of total triglycerides, total cholesterol, and HDL-C; the last analysis is performed after apoB-containing lipoproteins are removed from the plasma. The plasma LDL-C concentration is then calculated from these measurements by the Friedewald formula:

LDL cholesterol=total cholesterol−HDL−VLDL

where VLDL is calculated as triglycerides divided by 5. This formula assumes that cholesterol content of VLDL is about 20% of the plasma triglyceride level. It is reliable only when triglycerides are 4.5 mmol/L (400 mg/dL) or less. LDL-C concentrations calculated by this formula may be inaccurate in the presence of severe hypertriglyceridemia or when the triglyceride-to-cholesterol ratio of VLDL differs from the usual 4 : 1 ratio (as occurs in dysbetalipoproteinemia). Specialized laboratories can directly assay different lipoproteins by ultracentrifugation or nuclear magnetic resonance techniques. Direct measurement of LDL-C is also available in many clinical laboratories.
Non-HDL cholesterol (total cholesterol minus HDL cholesterol) is a measure of atherogenic lipoproteins that is useful when triglycerides are elevated and when patients are not fasting.

A triglyceride level higher than 11.3 mmol/L (1000 mg/dL) usually signifies the presence of two or more abnormalities of lipid metabolism (e.g., estrogen therapy in the presence of underlying familial hypertriglyceridemia). Elevated plasma triglyceride levels can fluctuate markedly in a single person over short periods. The fluctuation occurs because the LPL-mediated clearance mechanisms for triglyceride-rich particles become saturated at plasma triglyceride concentrations of approximately 5.6 mmol/L (500 mg/dL), and above this level plasma triglyceride concentrations largely reflect dietary fat intake. Therefore, triglyceride levels can rise precipitously as dietary fat intake increases and can fall rapidly with dietary fat restriction.

A complete plasma lipid profile (total cholesterol, LDL-C, HDL-C, and triglycerides) should be measured in all adults 20 years of age and older. It is reasonable to assess traditional atherosclerotic cardiovascular disease (ASCVD) risk factors every 4 to 6 years in adults 20 to 79 years of age who are free from ASCVD. In adults aged 40 to 79 without ASCVD, 10-year ASCVD risk can be estimated every 4 to 6 years. If the patient does not have an indication for LDL-lowering therapy, data support screening every 4 to 6 years between ages 40 and 75.Triglycerides should be measured in all patients with pancreatitis.

Lipid screening in children is controversial. The National Lipid Association Expert Panel recommended screening all children between the ages of 9 and 11 and as young as 2 years of age in the presence of known family history of hyperlipidemia or vascular disease. This approach is similar to that of a National Heart, Lung, and Blood Institute Expert Panel, which also included screening all children between ages 9 and 11 and again between ages 17 and 21 with earlier lipid testing in some children

because of high-risk conditions or family history of premature coronary artery disease.

Patient Selection and Treatment Goals

Prevention of ASCVD is the primary goal of the 2013 American College of Cardiology (ACC)/American Heart Association (AHA) Guidelines. These guidelines address risk assessment, lifestyle modifications,evaluation and treatment of obesity, and evaluation and management of blood cholesterol.

The 2013 ACC/AHA cholesterol guidelines define four groups qualifying for statin therapy: patients with clinical ASCVD, patients with LDL cholesterol 190 mg/dL or higher, patients with diabetes, and patients with a calculated ASCVD risk of 7.5% or more (Table 37-7). For patients without clinical ASCVD or LDL 190 mg/dL or higher, the guidelines advise calculating risk for ASCVD based on age, sex, ethnicity, total and HDL cholesterol, systolic blood pressure (treated or untreated), presence of diabetes mellitus, and current smoking status. A risk calculator with the guidelines applies to U.S. populations.176 Different risk scoring systems may be appropriate for diverse populations and regions.

Hyperlipidemia treatment in patients with established CHD is considered secondary prevention, whereas treatment in those who do not have known disease is primary prevention. When lipid-lowering therapy for primary prevention should be initiated is an unresolved question. There are no reliable biomarkers or imaging techniques that predict first events in people with dyslipidemia. Studies of high-risk groups for primary prevention have provided some guidance. The JUPITER trial153 showed a statistically significant reduction in total mortality ($p < 0.02$) in men aged 50 years and older and women 60 years and older, many of whom would not have fit the NCEP Adult Treatment Panel III guidelines for therapy. Treatment of hypercholesterolemia in persons older than 85 years is of unclear benefit, but CHD accounts for a high percentage of deaths in this age group, and there are survival benefits of treatment in elderly patients up to the age of 85 years who have known CHD. Guidelines for treatment of patients with type 2 diabetes mellitus take into account their increased risk of cardiovascular events. Patients with established vascular disease and diabetes mellitus are considered to be at very high risk.

Severe hypertriglyceridemia (>11.3 mmol/L [1000 mg/dL]) should be treated aggressively because pancreatitis associated with these levels can be fatal.167

The 2013 ACC/AHA cholesterol guidelines used clinical trial data to identify individuals for whom there is substantial evidence of the benefit of therapy. The guidelines do not specify treatment thresholds or goals of therapy, but other guidelines have included treatment goals as well as other risk scoring systems and calculators.The International Atherosclerosis Society recommends optimal levels of non-HDL cholesterol in addition to LDL cholesterol.

Some patients with LDL-C levels below previous treatment thresholds benefit from statin treatment. Therefore, treat-to-target approaches may not capture all patients who would benefit from therapy. Data from multiple sources suggest that LDL levels lower than those identified as treatment goals decrease the risk of atherosclerosis. Cardiovascular outcome trials using PCSK9 antibodies, which can reduce LDL-C to very low levels, may lead to recommendations for LDL-C levels below current goals.

ATHEROSCLEROSIS

A **Heart Attack** occurs when the blood flow that brings oxygen to the heart muscle is severely reduced or stopped. This happens because coronary arteries that supply the heart with blood can slowly become thicker and harder from a buildup of fat, cholesterol and other substances, called plaque. This slow process is known as atherosclerosis. If the plaque breaks open and a blood clot forms that blocks the blood flow, a heart attack occurs.

Arteriosclerosis occurs when the blood vessels that carry oxygen and nutrients from your heart to the rest of your body (arteries) become thick and stiff — sometimes restricting blood flow to your organs and tissues. Healthy arteries are flexible and elastic, but over time, the walls in your arteries can harden, a condition commonly called hardening of the arteries.

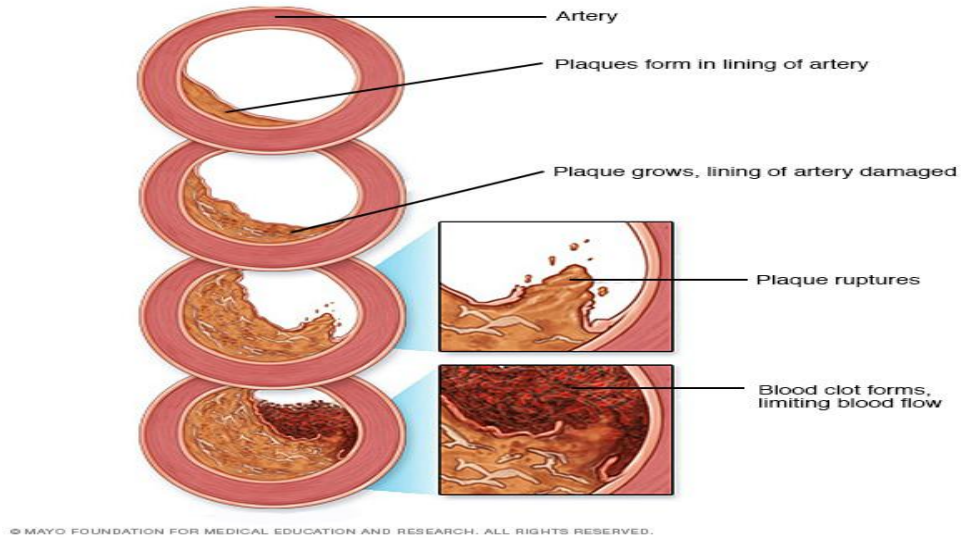
Atherosclerosis is a specific type of arteriosclerosis, but the terms are sometimes used interchangeably. Atherosclerosis refers to the buildup of fats, cholesterol and other substances in and on your artery walls (plaque), which can restrict blood flow.

The plaque can burst, triggering a blood clot. Although atherosclerosis is often considered a heart problem, it can affect arteries anywhere in your body. Atherosclerosis may be preventable and is treatable.

Symptoms

- **If you have atherosclerosis in your heart arteries,** you may have symptoms, such as chest pain or pressure (angina).
- **If you have atherosclerosis in the arteries leading to your brain,** you may have signs and symptoms such as sudden numbness or weakness in your arms or legs, difficulty speaking or slurred speech, temporary loss of vision in one eye, or drooping muscles in your face. These signal a transient ischemic attack (TIA), which, if left untreated, may progress to a stroke.
- **If you have atherosclerosis in the arteries in your arms and legs,** you may have symptoms of peripheral artery disease, such as leg pain when walking (claudication).
- **If you have atherosclerosis in the arteries leading to your kidneys,** you develop high blood pressure or kidney failure.

Causes



Development of atherosclerosis

Atherosclerosis is a slow, progressive disease that may begin as early as childhood. Although the exact cause is unknown, atherosclerosis may start with damage or injury to the inner layer of an artery. The damage may be caused by:

- High blood pressure
- High cholesterol
- High triglycerides, a type of fat (lipid) in your blood
- Smoking and other sources of tobacco
- Insulin resistance, obesity or diabetes
- Inflammation from diseases, such as arthritis, lupus or infections, or inflammation of unknown cause

Once the inner wall of an artery is damaged, blood cells and other substances often clump at the injury site and build up in the inner lining of the artery.

Over time, fatty deposits (plaque) made of cholesterol and other cellular products also build up at the injury site and harden, narrowing your arteries. The organs and tissues connected to the blocked arteries then don't receive enough blood to function properly.

Eventually, pieces of the fatty deposits may break off and enter your bloodstream.

In addition, the smooth lining of the plaque may rupture, spilling cholesterol and other substances into your bloodstream. This may cause a blood clot, which can block the blood flow to a specific part of your body, such as occurs when blocked blood flow to

your heart causes a heart attack. A blood clot can also travel to other parts of your body, blocking flow to another organ.

Risk factors

Hardening of the arteries occurs over time. Besides aging, factors that increase the risk of atherosclerosis include:

- High blood pressure
- High cholesterol
- Diabetes
- Obesity
- Smoking and other tobacco use
- A family history of early heart disease
- Lack of exercise
- An unhealthy diet

Complications

The complications of atherosclerosis depend on which arteries are blocked. For example:

- **Coronary artery disease.** When atherosclerosis narrows the arteries close to your heart, you may develop coronary artery disease, which can cause chest pain (angina), a heart attack or heart failure.
- **Carotid artery disease.** When atherosclerosis narrows the arteries close to your brain, you may develop carotid artery disease, which can cause a transient ischemic attack (TIA) or stroke.
- **Peripheral artery disease.** When atherosclerosis narrows the arteries in your arms or legs, you may develop circulation problems in your arms and legs called peripheral artery disease. This can make you less sensitive to heat and cold, increasing your risk of burns or frostbite. In rare cases, poor circulation in your arms or legs can cause tissue death (gangrene).

  Aneurysms. Atherosclerosis can also cause aneurysms, a serious complication that can occur anywhere in your body. An aneurysm is a bulge in the wall of your artery.

  Most people with aneurysms have no symptoms. Pain and throbbing in the area of an aneurysm may occur and is a medical emergency.

If an aneurysm bursts, you may face life-threatening internal bleeding. Although this is usually a sudden, catastrophic event, a slow leak is possible. If a blood clot within an aneurysm dislodges, it may block an artery at some distant point.

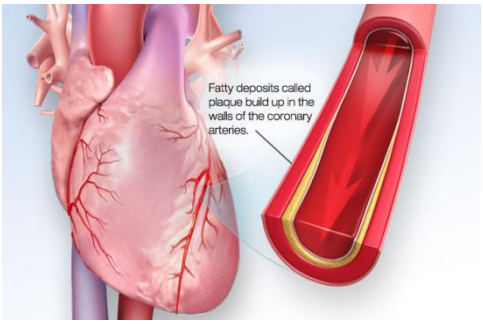
Chronic kidney disease. Atherosclerosis can cause the arteries leading to your kidneys to narrow, preventing oxygenated blood from reaching them. Over time, this can affect your kidney function, keeping waste from exiting your body.

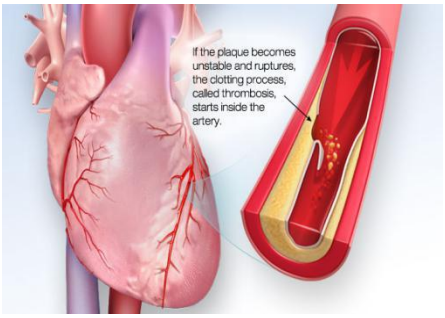
Prevention

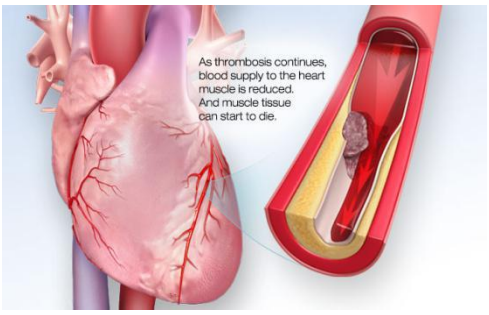
The same healthy lifestyle changes recommended to treat atherosclerosis also help prevent it. These include:

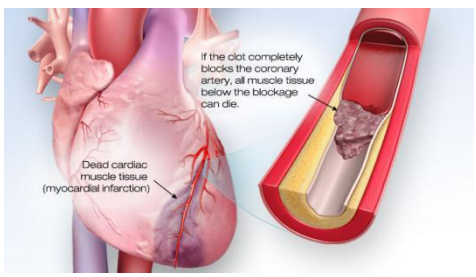
- Quitting smoking
- Eating healthy foods
- Exercising regularly
- Maintaining a healthy weight

Just remember to make changes one step at a time, and keep in mind what lifestyle changes are manageable for you in the long run.









Lipid Storage Diseases

Lipid storage diseases are a group of inherited metabolic disorders in which harmful amounts of fatty materials (lipids) accumulate in various tissues and cells in the body. Lipids are important parts of the membranes found within and between each cell and in the myelin sheath that coast and protects the nerves. Over time, this excessive storage of fats can cause permanent cellular and tissue damage, particularly in the brain, peripheral nervous system, liver, spleen, and bone marrow. Lipid storage diseases are inherited from one or both parents who carry a defective gene. Symptoms may appear early in life or develop in the teen or even adult years. Neurological complications of the lipid storage diseases may include ataxia, eye paralysis, brain degeneration, seizures, learning problems, spasticity, feeding and swallowing difficulties, slurred speech, loss of muscle tone, hypersensitivity to touch, pain in the arms and legs, and clouding of the cornea.

Treatment

Currently there is no specific treatment available for most of the lipid storage disorders, although Gaucher and Fabry diseases have highly effective enzyme replacement therapies. There is currently no cure for Niemann-Pick disease. Treatment is supportive. Doctors often prescribe corticosteroids to relieve the pain of Farber's disease. Anticonvulsant medications are often used to control seizures in Tay-Sachs disease.

Prognosis

The prognosis for a lipid storage disorder is determined by the type of disease, the age of onset, and the severity of symptoms. Children with Gaucher disease may live well into adulthood, while children with Niemann-Pick disease most often die at a young age from infection or progressive neurological loss. Children with Fabry disease

often die prematurely of complications from heart disease, renal failure, or stroke. Most children with Farber's disease die by age 2, usually from lung disease. Children with Tay-Sachs disease may eventually need a feeding tube and they often die by age 4 from recurring infection.

What is fatty liver disease?

Your liver is the largest organ inside your body. It helps your body digest food, store energy, and remove poisons. Fatty liver disease is a condition in which fat builds up in your liver. There are two main types:

Nonalcoholic fatty liver disease (NAFLD)

Alcoholic fatty liver disease, also called alcoholic steatohepatitis

What is nonalcoholic fatty liver disease (NAFLD)?

NAFLD is a type of fatty liver disease that is not related to heavy alcohol use. There are two kinds:

Simple fatty liver, in which you have fat in your liver but little or no inflammation or liver cell damage. Simple fatty liver typically does not get bad enough to cause liver damage or complications.

Nonalcoholic steatohepatitis (NASH), in which you have inflammation and liver cell damage, as well as fat in your liver. Inflammation and liver cell damage can cause fibrosis, or scarring, of the liver. NASH may lead to cirrhosis or liver cancer.

What is alcoholic fatty liver disease?

Alcoholic fatty liver disease is due to heavy alcohol use. Your liver breaks down most of the alcohol you drink, so it can be removed from your body. But the process of breaking it down can generate harmful substances. These substances can damage liver cells, promote inflammation, and weaken your body's natural defenses. The more alcohol that you drink, the more you damage your liver. Alcoholic fatty liver disease is the earliest stage of alcohol-related liver disease. The next stages are alcoholic hepatitis and cirrhosis.

Who is at risk for fatty liver disease?

The cause of nonalcoholic fatty liver disease (NAFLD) is unknown. Researchers do know that it is more common in people who

Have type 2 diabetes and prediabetes

Have obesity

Are middle aged or older (although children can also get it)

Are Hispanic, followed by non-Hispanic whites. It is less common in African Americans.

Have high levels of fats in the blood, such as cholesterol and triglycerides

Have high blood pressure

Take certain drugs, such as corticosteroids and some cancer drugs

Have certain metabolic disorders, including metabolic syndrome

Have rapid weight loss

Have certain infections, such as hepatitis C

Have been exposed to some toxins

NAFLD affects about 25 percent of people in the world. As the rates of obesity, type 2 diabetes, and high cholesterol are rising in the United States, so is the rate of NAFLD. NAFLD is the most common chronic liver disorder in the United States.

Alcoholic fatty liver disease only happens in people who are heavy drinkers, especially those who have been drinking for a long period of time. The risk is higher for heavy drinkers who are women, have obesity, or have certain genetic mutations.

What are the symptoms of fatty liver disease?

Both NAFLD and alcoholic fatty liver disease are usually silent diseases with few or no symptoms. If you do have symptoms, you may feel tired or have discomfort in the upper right side of your abdomen.

How is fatty liver disease diagnosed?

Because there are often no symptoms, it is not easy to find fatty liver disease. Your doctor may suspect that you have it if you get abnormal results on liver tests that you had for other reasons. To make a diagnosis, your doctor will use

Your medical history

A physical exam

Various tests, including blood and imaging tests, and sometimes a biopsy

As part of the medical history, your doctor will ask about your alcohol use, to find out whether fat in your liver is a sign of alcoholic fatty liver disease or nonalcoholic fatty liver (NAFLD). He or she will also ask which medicines you take, to try to determine whether a medicine is causing your NAFLD.

During the physical exam, your doctor will examine your body and check your weight and height. Your doctor will look for signs of fatty liver disease, such as

An enlarged liver

Signs of cirrhosis, such as jaundice, a condition that causes your skin and whites of your eyes to turn yellow

You will likely have blood tests, including liver function tests and blood count tests. In some cases you may also have imaging tests, like those that check for fat in the liver and the stiffness of your liver. Liver stiffness can mean fibrosis, which is scarring of the liver. In some cases you may also need a liver biopsy to confirm the diagnosis, and to check how bad the liver damage is.

What are the treatments for fatty liver disease?

Doctors recommend weight loss for nonalcoholic fatty liver. Weight loss can reduce fat in the liver, inflammation, and fibrosis. If your doctor thinks that a certain medicine is the cause of your NAFLD, you should stop taking that medicine. But

check with your doctor before stopping the medicine. You may need to get off the medicine gradually, and you might need to switch to another medicine instead.

There are no medicines that have been approved to treat NAFLD. Studies are investigating whether a certain diabetes medicine or Vitamin E can help, but more studies are needed.

The most important part of treating alcohol-related fatty liver disease is to stop drinking alcohol. If you need help doing that, you may want to see a therapist or participate in an alcohol recovery program. There are also medicines that can help, either by reducing your cravings or making you feel sick if you drink alcohol.

Both alcoholic fatty liver disease and one type of nonalcoholic fatty liver disease (nonalcoholic steato hepatitis) can lead to cirrhosis. Doctors can treat the health problems caused by cirrhosis with medicines, operations, and other medical procedures. If the cirrhosis leads to liver failure, you may need a liver transplant.

What are some lifestyle changes that can help with fatty liver disease?

If you have any of the types of fatty liver disease, there are some lifestyle changes that can help:Eat a healthy diet, limiting salt and sugar, plus eating lots of fruits, vegetables, and whole grains

Get vaccinations for hepatitis A and B, the flu and pneumococcal disease. If you get hepatitis A or B along with fatty liver, it is more likely to lead to liver failure. People with chronic liver disease are more likely to get infections, so the other two vaccinations are also important.

Get regular exercise, which can help you lose weight and reduce fat in the liver

Talk with your doctor before using dietary supplements, such as vitamins, or any complementary or alternative medicines or medical practices. Some herbal remedies can damage your liver.

DISORDERS OF METABOLISM - II

Amino acid metabolism disorders are hereditary metabolic disorders. Hereditary disorders occur when parents pass the defective genes that cause these disorders on to their children. In most hereditary metabolic disorders, both parents of the affected child carry a copy of the abnormal gene. Because usually two copies of the abnormal gene are necessary for the disorder to occur, usually neither parent has the disorder. Some hereditary metabolic disorders are X-linked, which means only one copy of the abnormal gene can cause the disorder in boys.

Amino acids are the building blocks of proteins and have many functions in the body. Hereditary disorders of amino acid processing (metabolism) can result from defects either in the breakdown of amino acids or in the body's ability to get amino acids into cells. Because these disorders cause symptoms early in life, newborns are routinely screened for several common amino acid disorders. In the United States, newborns are commonly screened for

Homocystinuria

Maple syrup urine disease

Phenylketonuria

Tyrosinemia

Newborns also are screened for a number of other inherited disorders, but screening varies from state to state.

Branched-Chain Amino Acids

The branched-chain amino acids are called "branched-chain" because of their chemical structure. Leucine, isoleucine, and valine are the branched-chain amino acids that are the building blocks of proteins in the body. If these amino acids are not properly metabolized, they and their toxic by-products build up in the blood and urine, causing certain disorders.

Isovaleric acidemia

When the amino acid leucine is not properly metabolized, harmful levels of isovaleric acid build up in the body. In isovaleric acidemia, the enzyme needed to break down leucine, called isovaleryl CoA dehydrogenase, is not present or not working correctly. Isovaleric acidemia is also known as sweaty feet syndrome, because accumulated isovaleric acid gives off an odor that smells like sweat.

There are two forms of isovaleric acidemia. One form manifests during the first few days of life, and the other form manifests several months or years after birth. Symptoms that occur in the first few days of life include poor feeding, vomiting, and breathing problems as infants develop a buildup of acid in the blood (metabolic acidosis), low blood sugar (hypoglycemia), and an increase in ammonia in the blood (hyperammonemia). Blood cells may not be made in the bone marrow the way they normally are. Symptoms in the form that manifests later in life come and go and are similar to the symptoms of the form that manifests earlier but are less severe.

Doctors diagnose isovaleric acidemia by doing tests of blood and urine to detect elevated levels of isovaleric acid.

To treat isovaleric acidemia, doctors give hydration and nutrition (including high doses of the sugar dextrose) by vein and glycine supplements to help the body get rid of the excess acid. If these measures do not help, doctors may need to remove small amounts of the infant's blood (one syringe at a time) and replace it with equal volumes of fresh donor blood (called exchange transfusion) and remove substances from the blood via a catheter inserted through the abdominal wall into the abdomen (called peritoneal dialysis). All affected people need to restrict their consumption of leucine and continue to take supplements of glycine and another amino acid called carnitine. The prognosis is excellent with treatment.

Maple syrup urine disease

Children with maple syrup urine disease are unable to metabolize leucine, isoleucine, and valine. By-products of these amino acids build up, causing neurologic changes, including seizures and intellectual disability. These by-products also cause body fluids and substances, such as urine, sweat, and earwax, to smell like maple syrup. This disease is most common among Mennonite families.

There are many forms of maple syrup urine disease. In the most severe form, infants have vomiting and lethargy and then develop neurologic abnormalities, including seizures and coma, during the first days of life and can die within days to weeks if untreated. In the milder forms, children initially appear normal, but during infection, surgery, or other physical stress, they can develop vomiting, staggering, confusion, and coma.

Since 2007, nearly every state in the United States has required that all newborns be screened for maple syrup urine disease with a blood test. Doctors also look for elevated levels of amino acids in the blood. The diagnosis is confirmed by genetic testing.

Doctors treat infants with severe disease by strictly limiting the diet and sometimes by removing substances from the blood via a catheter inserted through the abdominal wall into the abdomen (called peritoneal dialysis) or by using a machine outside the body to remove and purify blood from the body (called hemodialysis). Doctors also give hydration and nutrition by vein.

Some children with mild disease benefit from injections of vitamin B1 (thiamin). After the disease has been brought under control, children must always consume a special artificial diet that is low in leucine, isoleucine, and valine. Care providers should have an emergency plan in place for how to handle a sudden attack because it may result in a build-up of toxic substances in the blood and low blood sugar (called metabolic crisis). Sudden attacks are most often triggered by common infections.

A liver transplant cures this disease.

Methylmalonic acidemia

When a certain enzyme is not functional, harmful levels of methylmalonic acid build up in the body. This disorder may also be caused by a deficiency of vitamin B12 (cobalamin). The age at which symptoms start, symptoms, and treatment are similar to those of propionic acidemia except that doctors may give supplements of vitamin B12 instead of biotin.

Propionic acidemia

When a specific enzyme (a type of protein) called propionyl CoA carboxylase is not functional, harmful levels of propionic acid build up in the body.

In most affected infants, symptoms begin in the first days or weeks after birth and include poor feeding, vomiting, and breathing problems as the infants develop a buildup of acid in the blood (metabolic acidosis), low blood sugar (hypoglycemia), and an increase in ammonia in the blood (hyperammonemia). Seizures or coma may occur. Stressors, such as fasting, fever, or infection, may trigger an attack. Children who survive this disorder may have kidney problems, intellectual disability, neurologic abnormalities, and heart problems.

Doctors diagnose propionic acidemia by doing tests of blood and urine to detect elevated levels of propionic acid. The diagnosis is confirmed by measuring levels of propionyl CoA carboxylase in white blood cells or other tissue cells and/or by genetic testing.

To treat propionic acidemia, doctors give hydration and nutrition (including high doses of the sugar dextrose) by vein and restrict the infant's consumption of protein. If these measures do not help, doctors may need to remove substances from the blood via a catheter inserted through the abdominal wall into the abdomen (called peritoneal dialysis) or use a machine outside the body to remove and purify blood from the body (called hemodialysis). As children age, they need to continue dietary restrictions and may need to take carnitine supplements. Doctors often give children antibiotics because bacteria in their intestines can cause propionic acid to build up. Care providers should have an emergency plan in place for how to handle a sudden attack because it may result in a build-up of toxic substances in the blood and low blood sugar (called metabolic crisis).

ALKAPTONURIA

Alkaptonuria is a rare inherited disorder. It occurs when your body can’t produce enough of an enzyme called homogentisic dioxygenase (HGD). This enzyme is used to break down a toxic substance called homogentisic acid. When you don’t produce enough HGD, homogentisic acid builds up in your body.

The buildup of homogentisic acid causes your bones and cartilage to become discolored and brittle. This typically leads to osteoarthritis, especially in your spine and large joints. People with alkaptonuria also have urine that turns dark brown or black when it's exposed to air.

What Are the Symptoms of Alkaptonuria?

Dark stains on a baby's diaper are one of the earliest signs of alkaptonuria. There are few other symptoms during childhood.

Symptoms become more obvious as you age. Your urine may turn dark brown or black when it's exposed to air. By the time you reach your 20s or 30s, you may notice signs of early-onset osteoarthritis. For example, you may notice chronic stiffness or pain in your lower back or large joints.

Other symptoms of alkaptonuria include:

- dark spots in the sclera (white) of your eyes
- thickened and darkened cartilage in your ears
- blue speckled discoloration of your skin, particularly around sweat glands
- dark-colored sweat or sweat stains
- black earwax
- kidney stones and prostate stones
- arthritis (especially hip and knee joints)

Alkaptonuria can also lead to heart problems. The buildup of homogentisic acid causes your heart valves to harden. This can keep them from closing properly, resulting in aortic and mitral valve disorders. In severe cases, heart valve replacement may be necessary. The buildup also causes your blood vessels to harden. This raises your risk of high blood pressure.

What Causes Alkaptonuria?

Alkaptonuria is caused by a mutation on your homogentisate 1,2-dioxygenase (HGD) gene. It's an autosomally recessive condition. This means that both of your parents must have the gene in order to pass the condition on to you.

Alkaptonuria is a rare disease.

How Is Alkaptonuria Diagnosed?

Your doctor may suspect you have alkaptonuria if your urine turns dark brown or black when it's exposed to air. They may also test you for the condition if you develop early onset osteoarthritis.Your doctor can use a test called gas

chromatography to look for traces of homogentisic acid in your urine. They can also use DNA testing to check for the mutated HGD gene.
Family history is very useful in making a diagnosis of alkaptonuria. However, many people don't know they carry the gene. Your parents might be carriers without realizing it.

How Is Alkaptonuria Treated?

There's no specific treatment for alkaptonuria.
You may be put on a low-protein diet. Your doctor may also recommend large doses of ascorbic acid, or vitamin C, to slow down the accumulation of homogentisic acid in your cartilage. However, NORD warns that long-term use of vitamin C has generally proven ineffective for treating this condition.
Other treatments for alkaptonuria are focused on preventing and relieving possible complications, such as:arthritis,heart disease and kidney stones

What is cystinuria?

Cystinuria is an inherited disease that causes stones made ofthe amino acid cystine to form in the kidneys, bladder, and ureters. Inherited diseases are passed down from parents to children through a defect in their genes. To get cystinuria, a person must inherit the defect from both parents.
The defect in the gene causes cystine to accumulate inside the kidneys, which are the organs that help regulate what goes in and out of your bloodstream. The kidneys have many functions, including:

- reabsorbing essential minerals and proteins back into the body
- filtering the blood to remove toxic waste
- producing urine to expel waste from the body

In someone who has cystinuria, the amino acid cystine builds up and forms stones instead of going back into the bloodstream. These stones can get stuck in the kidneys, bladder, and ureters. This can be very painful until the stones pass through urination. Very large stones may need to be surgically removed.
The stones can recur many times. Treatments are available to manage pain and to prevent more stones from forming.

What are the symptoms of cystinuria?

Although cystinuria is a lifelong condition, symptoms typically first occur in young adults, according to a study in the European Journal of Urology. There have been rare cases in infants and adolescents. The symptoms may include:

- blood in the urine
- severe pain in the side or the back, almost always on one side
- nausea and vomiting
- pain near the groin, pelvis, or abdomen

Cystinuria is asymptomatic, meaning it causes no symptoms, when there are no stones. However, the symptoms will recur each time stones form in the kidneys. The stones commonly occur more than once.

What causes cystinuria?

Defects, also called mutations, in the genes SLC3A1 and SLC7A9 cause cystinuria. These genes provide the instructions for your body to make a certain transporter protein found in the kidneys. This protein normally controls the reabsorption of certain amino acids.

Amino acids are formed when the body digests and breaks down proteins. They're used to perform a wide variety of bodily functions, so they're important to your body and aren't considered waste. Therefore, when these amino acids enter the kidneys, they're normally absorbed back into the bloodstream. In people with cystinuria, the genetic defect interferes with the transporter protein's ability to reabsorb the amino acids.

One of the amino acids — cystine — isn't very soluble in urine. If it isn't reabsorbed, it will accumulate inside the kidney and form crystals, or cystine stones. The rock-hard stones then get stuck in the kidneys, bladder, and ureters. This can be very painful.

Who is at risk for cystinuria?

You're at risk of getting cystinuria only if your parents have the specific defect in their gene that causes the disease. As well, you only get the disease if you inherit the defect from both of your parents. Cystinuria occurs in about 1 in every 10,000 people around the world, so it's fairly rare.

How is cystinuria diagnosed?

Cystinuria is usually diagnosed when someone experiences an episode of kidney stones. A diagnosis is then made by testing the stones to see if they are made out of

cystine. Rarely is genetic testing done. Additional diagnostic testing could include the following:

24-hour urine collection

You will be asked to collect your urine in a container over the course of an entire day. The urine will then be sent to a laboratory for analysis.

Intravenous pyelogram

An X-ray examination of the kidneys, bladder, and ureters, this method uses a dye in the bloodstream to help see the stones.

Abdominal CT scan

This type of CT scan uses X-rays to create images of the structures inside the abdomen to look for stones inside the kidneys.

Urinalysis

This is an examination of urine in a laboratory that may involve looking at the color and physical appearance of the urine, viewing the urine under a microscope, and conducting chemical tests to detect certain substances, such as cystine.

What are the complications of cystinuria?

If not treated properly, cystinuria can be extremely painful and may lead to serious complications. These complications include:

- kidney or bladder damage from a stone
- urinary tract infections
- kidney infections
- ureteral obstruction, a blockage of the ureter, the tube that drains urine from the kidneys into the bladder

How is cystinuria treated? | Treatment

Changes to your diet, medications, and surgery are options for treating the stones that form due to cystinuria.

Dietary changes

Reducing salt intake to less than 2 grams per day has also been shown to be helpful in preventing stone formation, according to a study in the European Journal of Urology.

Adjusting pH balance

Cystine is more soluble in urine at a higher pH, which is a measure of how acidic or basic a substance is. Alkalinizing agents, such as potassium citrate or acetazolamide, will increase the pH of urine to make cystine more soluble. Some alkalinizing medications can be purchased over the counter. You should talk to your doctor before taking any type of supplement.

Medications

Medications known as chelating agents will help to dissolve cystine crystals. These drugs work by chemically combining with the cystine to form a complex that can then dissolve in urine. Examples include D-penicillamine and alpha-mercaptopropionylglycine. D-penicillamine is effective, but it has many side effects.

Pain medications may also be prescribed to control pain while the stones pass through the bladder and out of the body.

Surgery

If the stones are very large and painful, or block one of the tubes leading from the kidney, they might need to be removed surgically. There are a few different types of surgeries to break up the stones. These include the following procedures:

- **Extracorporeal shock wave lithotripsy (ESWL)**:This procedure uses shock waves to break up large stones into smaller pieces. It's not as effective for cystine stones as for other types of kidney stones.
- **Percutaneous nephrostolithotomy** (or **nephrolithotomy**): This procedure involves passing a special instrument through your skin and into your kidney to take out the stones or break them apart.

ALBINISM

Albinism is a rare group of genetic disorders that cause the skin, hair, or eyes to have little or no color. Albinism is also associated with vision problems. According to the National Organization for Albinism and Hypopigmentation, about 1 in 18,000 to 20,000 people in the United States have a form of albinism.

What are the types of albinism?

Different gene defects characterize the numerous types of albinism. Types of albinism include:

Oculocutaneous albinism (OCA)

OCA affects the skin, hair, and eyes. There are several subtypes of OCA:

OCA1

OCA1 is due to a defect in the tyrosinase enzyme. There are two subtypes of OCA1:

- **OCA1a.** People with OCA1a have a complete absence of melanin. This is the pigment that gives skin, eyes, and hair their coloring. People with this subtype have white hair, very pale skin, and light eyes.
- **OCA1b.** People with OCA1b produce some melanin. They have light-colored skin, hair, and eyes. Their coloring may increase as they age.

OCA2

OCA2 is less severe than OCA1. It's due to a defect in the OCA2 gene that results in reduced melanin production. People with OCA2 are born with light coloring and skin. Their hair may be yellow, blond, or light brown. OCA2 is most common in people of African descent and Native Americans.

OCA3

OCA3 is a defect in the TYRP1 gene. It usually affects people with dark skin, particularly Black South Africans. People with OCA3 have reddish-brown skin, reddish hair, and hazel or brown eyes.

OCA4

OCA4 is due to a defect in the SLC45A2 protein. It results in a minimal production of melanin and commonly appears in people of East Asian descent. People with OCA4 have symptoms similar to those in people with OCA2.

Ocular albinism

Ocular albinism is the result of a gene mutation on the X chromosome and occurs almost exclusively in males. This type of albinism only affects the eyes. People with this type have normal hair, skin, and eye coloring, but have no coloring in the retina (the back of the eye).

Hermansky-Pudlak syndrome

This syndrome is a rare form of albinism that's due to a defect in one of eight genes. It produces symptoms similar to OCA. The syndrome occurs with lung, bowel, and bleeding disorders.

Chediak-Higashi syndrome

Chediak-Higashi syndrome is another rare form of albinism that's the result of a defect in the LYST gene. It produces symptoms similar to OCA, but may not affect all areas of the skin. Hair is usually brown or blond with a silvery sheen. The skin is usually creamy white to grayish. People with this syndrome have a defect in the white blood cells, increasing their risk of infections.

Griscelli syndrome

Griscelli syndrome is an extremely rare genetic disorder. It's due to a defect in one of three genes. There only have been 60 known casesTrusted Source of this syndrome worldwide since 1978. It occurs with albinism (but may not affect the entire body), immune problems, and neurological problems. Griscelli syndrome usually results in death within the first decade of life.

What causes albinism?

A defect in one of several genes that produce or distribute melanin causes albinism. The defect may result in the absence of melanin production, or a reduced amount of melanin production. The defective gene passes down from both parents to the child and leads to albinism.

Who's at risk for albinism?

Albinism is an inherited disorder that's present at birth. Children are at risk of being born with albinism if they have parents with albinism, or parents who carry the gene for albinism.

What are the symptoms of albinism?

People with albinism will have the following symptoms:

- an absence of color in the hair, skin, or eyes
- lighter than normal coloring of the hair, skin, or eyes
- patches of skin that have an absence of color

Albinism occurs with vision problems, which may include:

- strabismus (crossed eyes)
- photophobia (sensitivity to light)
- nystagmus (involuntary rapid eye movements)
- impaired vision or blindness
- astigmatism

How is albinism diagnosed?

The most accurate way to diagnose albinism is through genetic testing to detect defective genes related to albinism. Less accurate ways of detecting albinism include an evaluation of symptoms by your doctor or an electroretinogram test. This test measures the response of the light-sensitive cells in the eyes to reveal eye problems associated with albinism.

What are the treatments for albinism?

There's no cure for albinism. However, treatment can relieve symptoms and prevent sun damage. Treatment may include:

- sunglasses to protect the eyes from the sun's ultraviolet (UV) rays
- protective clothing and sunscreen to protect the skin from UV rays
- prescription eyeglasses to correct vision problems
- surgery on the muscles of the eyes to correct abnormal eye movements

Diseases due to Errors in Nucleic Acid Metabolism

The following points highlight the six major diseases caused due to errors in nucleic acid metabolism. The diseases are: 1. Lesch-Nyhan Syndrome 2. Hereditary Xanthinuria 3. Orotic Aciduria 4. Hypouricemia 5. Von Gierke's Disease 6. Reye's Syndrome.

Errors in Nucleic Acid Metabolism: Disease

 # 1. Lesch-Nyhan Syndrome:

a. This condition is characterized by the complete deficiency of phosphoribosyl transferase, which causes hypoxanthine or guanine to form a nucleotide with PRPP (5′- phosphoribosyl – 1′- pyrophosphate). These purines are thus available for the formation of uric acid.

b. This disorder is X-linked in its inheritance

c. This appears in childhood as a severe neurological syndrome, which is sometimes accompanied by gout. The urinary uric acid amount is five to six times the normal.

d. Hypothyroidism, hypo- and hyperparathyroidism are accompanied by hyperuricemia.

e. Hypertension is accompanied by increased plasma uric acid. Such patients show an increased tendency to myocardial infarction, which is also a cause of hyperuricemia.

f. It can be prevented or diminished by the administration of allopurinol, an analogue of hypoxanthine. Allopurinol inhibits xanthine oxidase due to which uric acid cannot be formed.

Errors in Nucleic Acid Metabolism: Disease

 # 2. Hereditary Xanthinuria:

a. In this rare genetic disorder, there is the deficiency of xanthine oxidase which leads to the diminished level of blood uric acid (1 mg/100 ml or less).

b. The urinary excretion contains large amounts of xanthine with lesser amounts of hypoxanthine.

c. Urinary calculi composed of xanthine may be produced.

Errors in Nucleic Acid Metabolism: Disease

 # 3. Orotic Aciduria:

a. This is an inherited disorder which causes the excessive production of orotic acid. This occurs by the deficiency of orotatc phosphoribosyl transferase.

b. The urinary excretion consists of large amounts of pyrimidine nucleotide precursor.

c. The urine becomes cloudy on cooling with the deposition of needle-shaped crystals of orotic acid.
d. Children affected by this condition develop a severe megaloblastic anemia with physical and mental retardation.
e. Administration of uridine improves this condition significantly.

Errors in Nucleic Acid Metabolism: Disease

 # 4. Hypouricemia

a. This is due to a genetic defect or to severe liver damage.
b. The deficiency of the enzyme xanthine oxidase causes hypouricemia and increased excretion of hypoxanthine and xanthine.
c. Patients may exhibit Xanthinuria and Xanthine lithiasis in severe xanthine oxidase deficiency.

Errors in Nucleic Acid Metabolism: Disease #

5. Von Gierke's Disease:

a. This disease is associated with the purine over-production and hyperuricemia.
b. There is also enhanced generation of the PRPP.
c. The associated lactic acidosis elevates the renal threshold for urate, increasing total body urates. (The rest is already mentioned in Glycogen storage disease. Add this to it).

Errors in Nucleic Acid Metabolism: Disease #

6. Reye's Syndrome:

a. The orotic aciduria accompanies this syndrome.
b. This syndrome exhibits inability of severely damaged mitochondria to use carbamoyl phosphate which is available for cystosotic over- production of orotic acid.

GOUT:

Gout is a common type of arthritis that causes intense pain, swelling, and stiffness in a joint. It usually affects the joint in the big toe.

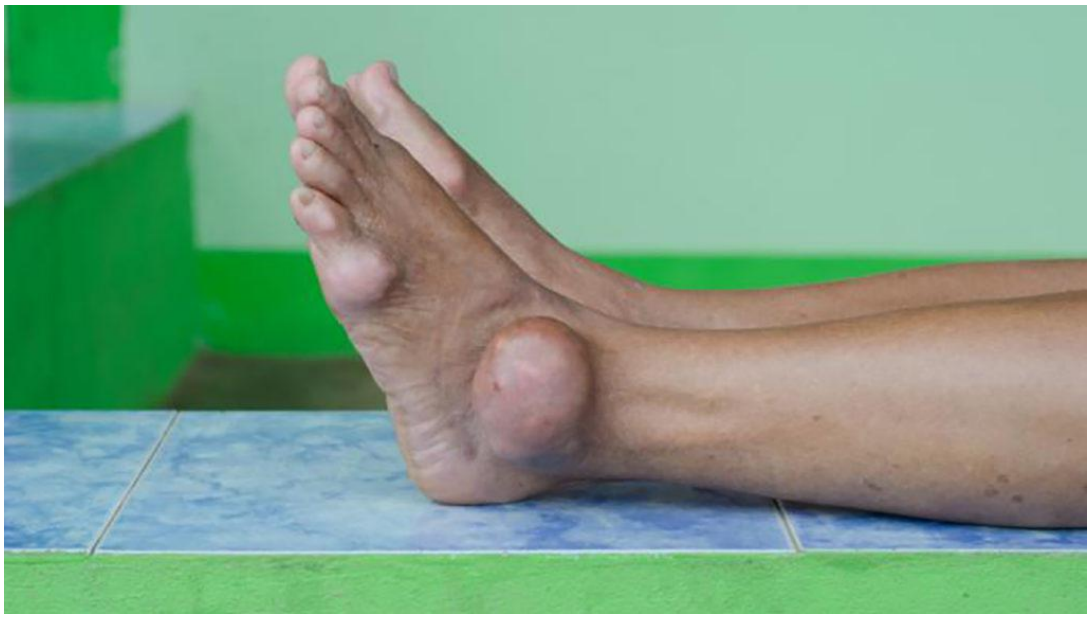
Gout attacks can come on quickly and keep returning over time, slowly harming tissues in the region of the inflammation, and can be extremely painful. Hypertension, cardiovascular, and obesity are risk factors for gout.

It is the most common form of inflammatory arthritis in men, and although it is more likely to affect men, women become more susceptible to it after the menopause.

Fast facts on gout

- Gout is a form of arthritis caused by excess uric acid in the bloodstream.
- The symptoms of gout are due to the formation of uric acid crystals in the joints and the body's response to them.
- Gout most classically affects the joint in the base of the big toe.
- Gout attacks often occur without warning in the middle of the night.
- Most gout cases are treated with specific medications.

Treatment



Share on PinterestGout patients often have acute inflammation around their joints.

The majority of gout cases are treated with medication. Medication can be used to treat the symptoms of gout attacks, prevent future flares, and reduce the risk of gout complications such as kidney stones and the development of tophi.

Commonly used medications include nonsteroidal anti-inflammatory drugs (NSAIDs), colchicine, or corticosteroids. These reduce inflammation and pain in the areas affected by gout and are usually taken orally.

Medications can also be used to either reduce the production of uric acid (xanthine oxidase inhibitors such as allopurinol) or improve the kidney's ability to remove uric acid from the body (probenecid).

Without treatment, an acute gout attack will be at its worst between 12 and 24 hours after it began. A person can expect to recover within 1 to 2 weeks without treatment, but there may be significant pain during this period.

Tests and diagnosis

Gout can be tricky to diagnose, as its symptoms, when they do appear, are similar to those of other conditions. While hyperuricemia occurs in the majority of people that

develop gout, it may not be present during a flare. On top of that, the majority of people with hyperuricemia do not develop gout.

One diagnostic test that doctors can carry out is the joint fluid test, where fluid is extracted from the affected joint with a needle. The fluid is then examined to see if any urate crystals are present.

As joint infections can also cause similar symptoms to gout, a doctor can look for bacteria when carrying out a joint fluid test in order to rule a bacterial cause. The fluid may need to be sent to a lab, where it can take several days to analyze.

Doctors can also do a blood test to measure the levels of uric acid in the blood, but, as mentioned, people with high uric acid levels do not always experience gout. Equally, some people can develop the symptoms of gout without having increased levels of uric acid in the blood.

Finally, doctors can search for urate crystals around joints or within a tophus using ultrasound scan. X-rays cannot detect gout, but may be used to rule out other causes.

Types

There are various stages through which gout progresses, and these are sometimes referred to as different types of gout.

Asymptomatic hyperuricemia

It is possible for a person to have elevated uric acid levels without any outward symptoms. At this stage, treatment is not required, though urate crystals may deposit in tissue and cause slight damage.

People with asymptomatic hyperuricemia may be advised to take steps to address any possible factors contributing to uric acid build-up.

Acute gout

This stage occurs when the urate crystals that have been deposited suddenly cause acute inflammation and intense pain. This sudden attack is referred to as a “flare” and will normally subside within 3 to 10 days. Flares can sometimes be triggered by stressful events, alcohol and drugs, as well as cold weather.

Interval or intercritical gout

This stage is the period in between attacks of acute gout. Subsequent flares may not occur for months or years, though if not treated, over time, they can last longer and occur more frequently. During this interval, further urate crystals are being deposited in tissue.

Chronic tophaceous gout

Chronic tophaceous gout is the most debilitating type of gout. Permanent damage may have occurred in the joints and the kidneys. The patient can suffer from chronic arthritis and develop tophi, big lumps of urate crystals, in cooler areas of the body such as the joints of the fingers.

It takes a long time without treatment to reach the stage of chronic tophaceous gout – around 10 years. It is very unlikely that a patient receiving proper treatment would progress to this stage.

Pseudogout

One condition that is easily confused with gout is pseudogout. The symptoms of pseudogout are very similar to those of gout, although thr flare-ups are usually less severe.

The major difference between gout and pseudogout is that the joints are irritated by calcium pyrophosphate crystals rather than urate crystals. Pseudogout requires different treatment to gout.

Causes

Gout is caused initially by an excess of uric acid in the blood, or hyperuricemia. Uric acid is produced in the body during the breakdown of purines – chemical compounds that are found in high amounts in certain foods such as meat, poultry, and seafood.

Normally, uric acid is dissolved in the blood and is excreted from the body in urine via the kidneys. If too much uric acid is produced, or not enough is excreted, it can build up and form needle-like crystals that trigger inflammation and pain in the joints and surrounding tissue.

Risk factors

There are a number of factors that can increase the likelihood of hyperuricemia, and therefore gout:

Age and gender: Men produce more uric acid than women, though women's levels of uric acid approach those of men after the menopause.

Genetics: A family history of gout increases the likelihood of the condition developing.

Lifestyle choices: Alcohol consumption interferes with the removal of uric acid from the body. Eating a high-purine diet also increases the amount of uric acid in the body.

Lead exposure: Chronic lead exposure has been linked to some cases of gout.

Medications: Certain medications can increase the levels of uric acid in the body; these include some diuretics and drugs containing salicylate.

Weight: Being overweight increases the risk of gout as there is more turnover of body tissue, which means more production of uric acid as a metabolic waste product. Higher levels of body fat also increase levels of systemic inflammation as fat cells produce pro-inflammatory cytokines.

Recent trauma or surgery: Increases risk.

Other health problems: Renal insufficiency and other kidney problems can reduce the body's ability to efficiently remove waste products, leading to elevated uric acid levels. Other conditions associated with gout include high blood pressure and diabetes.

Symptoms

Gout usually becomes symptomatic suddenly without warning, often in the middle of the night.

The main symptoms are intense joint pain that subsides to discomfort, inflammation, and redness.

Gout frequently affects the large joint of the big toe, but can also affect the forefoot, ankles, knees, elbows, wrists, and fingers.

The pain can be excruciating. A veteran visiting a Hospital in Birmingham, AL, said:

> "I've been shot, beat up, stabbed, and thrown out of a helicopter, but none of that compared to the gout."

Complications

In some cases, gout can develop into more serious conditions, such as:

- Kidney stones: If urate crystals collect in the urinary tract, they can become kidney stones.
- Recurrent gout: Some people only ever have one flare up; others may have regular recurrences, causing gradual damage to the joints and surrounding tissue.

Prevention tips

There are many lifestyle and dietary guidelines that can be tried to protect against flares or prevent gout from occurring in the first instance:

- maintain a high fluid intake of around 2 to 4 liters a day
- avoid alcohol
- maintain a healthy body weight

Home remedies

Individuals with gout can manage flare-ups by moderating their diet. A balanced diet can help reduce symptoms.

Decreasing foods that are high in purines, to ensure that the levels of uric acid in the blood do not get too high, is reasonable to try. Here is a list of high-purine foods to be wary of:

- anchovies
- asparagus
- beef kidneys
- brains
- dried beans and peas
- game meats
- gravy
- herring
- liver
- mackerel
- mushrooms
- sardines
- scallops
- sweetbreads

While it is reasonable to decrease or avoid these foods, it has been found that a high purine-rich diet does not increase the risk of gout, or aggrevate symptoms in research studies.

Asparagus, beans, some other plant-based foods, and mushrooms are also sources of purines, but research suggests that these do not trigger gout attacks and do not impact uric acid levels.

Various epidemiological studies have shown that purine-rich vegetables, whole grains, nuts and legumes, and less sugary fruits, coffee, and vitamin C supplements lower blood uric acid levels, but do not decrease the risk of gout. Red meat, fructose-containing beverages, and alcohol can increase the risk.

The role of uric acid in gout has been clearly defined and understood. As a result of this and the wide availability of relevant medications, gout is a very controllable form of arthritis.

Disorders in bilirubin metabolism

Biliary excretion is the main route of disposal of bilirubin and impaired excretion results in jaundice, a well recognisable symptom of liver disease. Bilirubin glucuronides are the major pigments in bile. In 1864, Georg Stadeler obtained a defined and pure preparation of the dark red bile pigment, which he named bilirubin. He also realised that the yellow colour in jaundiced patients was caused by accumulation of bilirubin. Furthermore, based on his estimations of their atomic compositions, he speculated that bilirubin, biliverdin and haematoidin, the blood pigment, are structurally related. The first convincing evidence that bile pigments indeed are derived from blood pigments. He showed that intravenous injection of blood pigments led to an increased production of bile pigments. Using the diazoprobe for bilirubin developed by Ehrlich, Hijmans van den Bergh and Muller showed that there are, in fact, two kinds of bilirubin in man direct reacting bilirubin, the only form present in bile, and indirect reacting that only reacted in the presence of a catalyst (alcohol), the main form in blood. Obstruction of bile flow results in the appearance of direct reacting bilirubin in blood. In 1956, three groups independently showed that direct bilirubin could be converted to indirect bilirubin by glucuronidase. Thus, it became clear that bilirubin was converted to bilirubin glucuronic acid in the liver and excreted in bile. After Dutton and Storey isolated uridine diphosphate glucuronosyltransferase (UDP)-glucuronic acid and showed that this was the glucuronic acid donor in glucuronidation reactions, bilirubin glucuronidation was reconstituted in vitro. The hepatic enzyme that catalysed the glucuronidation of bilirubin was named bilirubin UDP-glucuronosyltransferase (B-UGT).The first paper about the similar structure of bilirubin and blood pigments was written by Rudolf Virchow in 1847. In the first issue of the journal that he founded, Virchows Archiv fur pathologische Anatomie und klinishe Medicin, he described presence of red

crystals in fluids from old haemorrhages that reminded him of bile pigment crystals. The theory of Virchow was confirmed by Fisher and co-workers in 1923 who proved the identity of crystalline bilirubin and haematoidin by crystallography]. In 1933, the same group disclosed the chemical structure of bilirubin and they were also the first to synthesise bilirubin in 1941. Although it was known since 1874 that bilirubin was derived from haeme, the exact mechanism was elucidated by Tenhunen and co-workers in 1968, who characterised haeme-oxygenase, the microsomal enzyme catalysing the formation of biliverdin from haeme. Two years later, this group also identified the cytosolic enzyme biliverdin reductase, catalysing the conversion of biliverdin to bilirubin. The complete scheme of the biosynthesis of bilirubin is shown in Fig. 1.

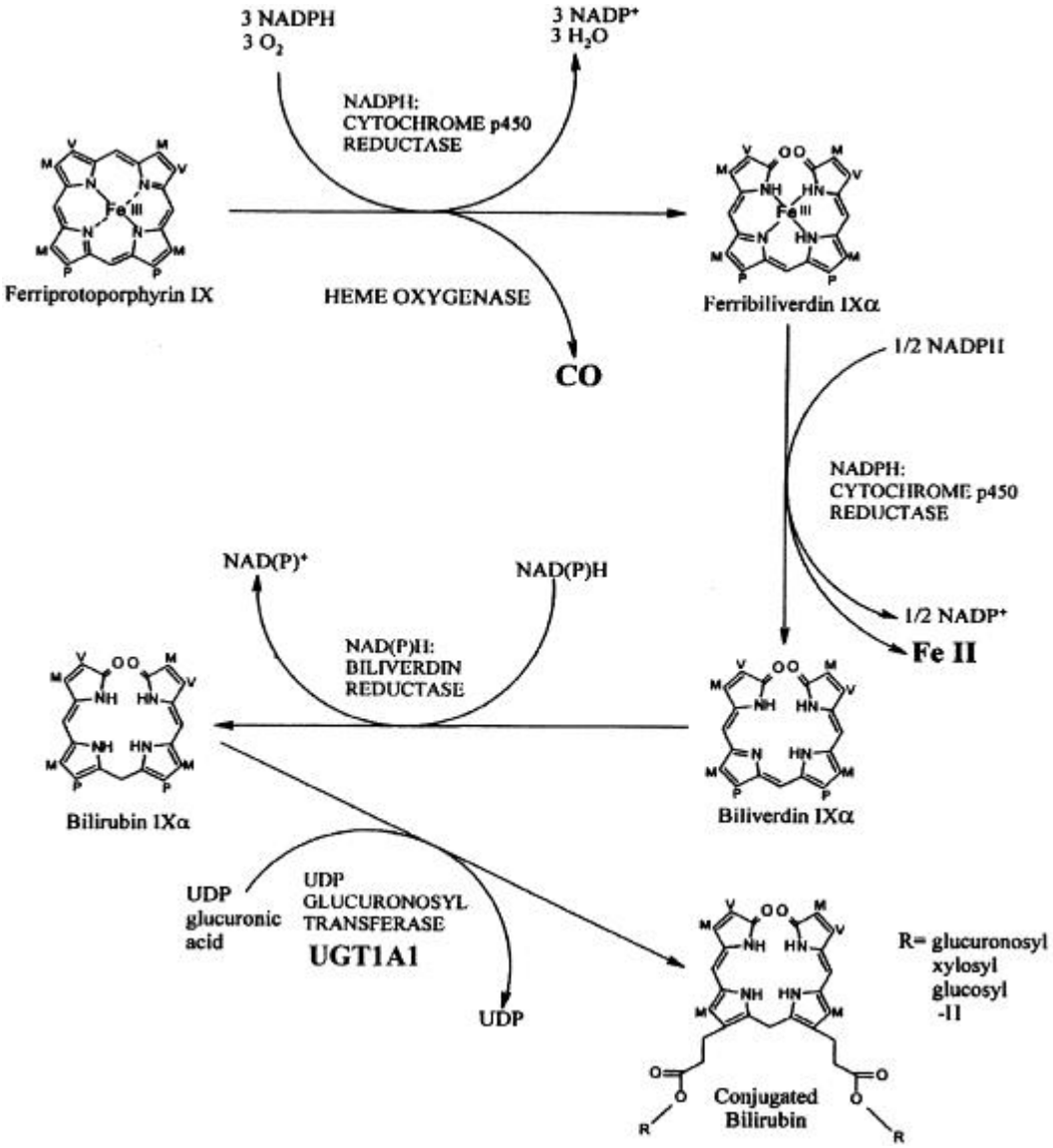


Fig. 1Bilirubin metabolism. The generation of bilirubin from haeme by haeme-oxygenase and subsequently biliverdin reductase. Glucuronidation of bilirubin by UDP-glucuronosyltransferase UGT1A1 results in the formation of bilirubin mono- and di-glucuronide which are excreted into bile.

2. Inherited forms of unconjugated hyperbilirubinemia

Three grades of inherited unconjugated hyperbilirubinemia are recognised in man. The mildest form, Gilbert syndrome, was described already in 1901 by Gilbert and Lereboulet. Since Gilbert syndrome is very common (and pubmed did not exist), other groups have rediscovered this syndrome. Meulengracht, for instance, published a paper entitled ‘A review of chronic intermittent juvenile jaundice’ in 1946 in which he summarises several of these early reports. A more recent report was published by Arias et al. in 1962. In contrast to the benign Gilbert syndrome, both severe forms of inherited unconjugated hyperbilirubinemia are very rare, affecting one in 1×106 new-borns.Crigler and Najjar described the first seven patients with severe forms of congenital non-haemolytic jaundice in 1952. Of these patients, six died in early childhood with kernicterus. Since their initial report, this lethal inherited disorder is called Crigler–Najjar (CN) syndrome. In 1969, Arias et al. studied bilirubin glucuronide excretion in bile and the response to phenobarbital in 16 patients

with CN syndrome and concluded that there were in fact two forms; CN type I and type II CN type I patients did not respond to phenobarbital and had no traces of bilirubin glucuronides in bile. This form was usually lethal in childhood. CN type II do respond to phenobarbital treatment and have traces of bilirubin glucuronides in bile. Although some patients with type II also died of kernicterus, most do survive into adulthood. The less severe hyperbilirubinemia and the presence of bilirubin glucuronides in bile indicated that type II patients do have some residual bilirubin glucuronidating activity while in type I patients, this is completely absent.In the elucidation of the molecular basis and pathophysiology of CN syndrome, the Gunn rat has proven to be a useful animal model. Gunn had already described this rat with unconjugated hyperbilirubinemia in 1938 . By injection of conjugated bilirubin, Axelroth showed that secretion of bilirubin glucuronide was not impaired in the Gunn rat, suggesting that high serum bilirubin levels were caused by a defect in bilirubin glucuronidation. Using an in vitro glucuronidation assay, it was shown that B-UGT indeed was absent from Gunn rat liver Because the glucuronidation of other substrates in the Gunn rat appeared normal, it was suspected that several UGT isoforms are present in mammals. Indeed, several UGT isoforms, which differed in substrate specificity, could be purified from rat liverThus, although it had become clear that CN and Gilbert syndrome were caused by a deficiency in hepatic glucuronidation of bilirubin, clarification of their genetic background had to await the identification of the gene encoding B-UGT, the UGT1A1 gene.

Jaundice:- classification, clinical features.

Jaundice is a term used to describe a yellowish tinge to the skin and the whites of the eye. Body fluids may also be yellow.The color of the skin and whites of the eyes will vary depending on levels of bilirubin. Bilirubin is a waste material found in the blood.

Moderate levels lead to a yellow color, while very high levels will appear brown.

About 60 percent of all infants born in the United States have jaundice. However, jaundice can happen to people of all ages and is normally the result of an underlying condition. Jaundice normally indicates a problem with the liver or bile duct.

In this article, Medical News Today will discuss what jaundice is, why it happens, and how it is diagnosed and treated.

Fast facts on jaundice

- Jaundice is caused by a buildup of bilirubin, a waste material, in the blood.
- An inflamed liver or obstructed bile duct can lead to jaundice, as well as other underlying conditions.
- Symptoms include a yellow tinge to the skin and whites of the eyes, dark urine, and itchiness.
- Diagnosis of jaundice can involve a range of tests.
- Jaundice is treated by managing the underlying cause.

Causes

Jaundice is a yellowing of the skin and the whites of eyes that happens when the body does not process bilirubin properly. This may be due to a problem in the liver.

It is also known as icterus.

Bilirubin is a yellow-colored waste material that remains in the bloodstream after iron is removed from the blood.

The liver filters waste out from the blood. When bilirubin reaches the liver, other chemicals attach to it. A substance called conjugated bilirubin results.

The liver produces bile, a digestive juice. Conjugated bilirubin enters the bile, then it leaves the body. It is this type of bilirubin that gives feces its brown color.

If there is too much bilirubin, it can leak into the surrounding tissues. This is known as hyperbilirubinemia, and it causes the yellow color in the skin and eyes.

Risk factors

Jaundice most often happens as a result of an underlying disorder that either causes the production of too much bilirubin or prevents the liver from getting rid of it. Both of these result in bilirubin being deposited in tissues.

Underlying conditions that may cause jaundice include:

- **Acute inflammation of the liver:** This may impair the ability of the liver to conjugate and secrete bilirubin, resulting in a buildup.
- **Inflammation of the bile duct:** This can prevent the secretion of bile and removal of bilirubin, causing jaundice.
- **Obstruction of the bile duct:** This prevents the liver from disposing of bilirubin.
- **Hemolytic anemia:** The production of bilirubin increases when large quantities of red blood cells are broken down.
- **Gilbert's syndrome:** This is an inherited condition that impairs the ability of enzymes to process the excretion of bile.
- **Cholestasis:** This interrupts the flow of bile from the liver. The bile containing conjugated bilirubin remains in the liver instead of being excreted.

Rarer conditions that may cause jaundice include:

- **Crigler-Najjar syndrome:** This is an inherited condition that impairs the specific enzyme responsible for processing bilirubin.

- **Dubin-Johnson syndrome:** This is an inherited form of chronic jaundice that prevents conjugated bilirubin from being secreted from of the cells of the liver.
- **Pseudojaundice:** This is a harmless form of jaundice. The yellowing of the skin results from an excess of beta-carotene, not from an excess of bilirubin. Pseudojaundice usually arises from eating large quantities of carrot, pumpkin, or melon.

Treatment

Treatment will depend on the underlying cause.

Jaundice treatment targets the cause rather than the jaundice symptoms.

The following treatments are used:

- Anemia-induced jaundice may be treated by boosting the amount of iron in the blood by either taking iron supplements or eating more iron-rich foods. Iron supplements are available for purchase online.
- Hepatitis-induced jaundice requires antiviral or steroid medications.
- Doctors can treat obstruction-induced jaundice by surgically removing the obstruction.
- If the jaundice has been caused by use of a medication, treatment for involves changing to an alternative medication.

Prevention

Jaundice is related to liver function. It is essential that people maintain the health of this vital organ by eating a balanced diet, exercising regularly, and not consuming more than the recommended amounts of alcohol.

Symptoms

Common symptoms of jaundice include:

- a yellow tinge to the skin and the whites of the eyes, normally starting at the head and spreading down the body
- pale stools
- dark urine
- itchiness

Accompanying symptoms of jaundice resulting from low bilirubin levels include:

- fatigue
- abdominal pain

- weight loss
- vomiting
- fever
- pale stools
- dark urine

Complications

The itching that accompanies jaundice can sometimes be so intense that patients have been known to scratch their skin raw, experience insomnia, or, in extreme cases, even have thoughts of suicide.

When complications happen, this is usually because of the underlying problem, not the jaundice itself.

For example, if an obstructed bile duct leads to jaundice, uncontrolled bleeding may result. This is because the blockage leads a shortage of vitamins needed for clotting.

Types

There are three main types of jaundice:

- Hepatocellular jaundice occurs as a result of liver disease or injury.
- Hemolytic jaundice occurs as a result of hemolysis, or an accelerated breakdown of red blood cells, leading to an increase in production of bilirubin.
- Obstructive jaundice occurs as a result of an obstruction in the bile duct. This prevents bilirubin from leaving the liver

Newborns

Jaundice is a common health issue in newborn infants. Around 60 percent of newborns experience jaundice, and this increases to 80 percent of premature infants born before 37 weeks of pregnancy.

They will normally show signs within 72 hours of birth.

Red blood cells in the body of an infant are frequently broken down and replaced. This causes the production of more bilirubin. Also, the livers of infants are less developed and, therefore, less effective at filtering bilirubin from the body.

Symptoms will usually resolve without treatment within 2 weeks. However, infants with extremely high bilirubin levels will require treatment with either a blood transfusion or phototherapy.

In these cases, treatment is vital as jaundice in newborns can lead to kernicterus, a very rare type of permanent brain damage.

Levels

The level of bilirubin is defined in a blood test called a bilirubin test. This measures unconjugated, or indirect, bilirubin levels. These are responsible for the onset of jaundice.

Bilirubin levels are measured in milligrams per decilitre (mg/dL). Adults and older children should have a level of between 0.3 and 0.6 mg/dL. Around 97 percent of infants born after 9 months of pregnancy have levels lower than 13 mg/dL. If they show higher levels than this, they are usually referred for further investigation.

These ranges may differ between laboratories. How far above the normal range a person's levels are will set out a course of treatment.

Diagnosis

Doctors will most likely use the history of the patient and a physical exam to diagnose jaundice and confirm bilirubin levels. They will pay close attention to the abdomen, feel for tumors, and check the firmness of the liver.

A firm liver indicates cirrhosis, or scarring of the liver. A rock-hard liver suggests cancer.

Several tests can confirm jaundice. The first is a liver function test to find out whether or not the liver is functioning properly.

If a doctor cannot find the cause, a doctor may request blood tests to check bilirubin levels and the composition of the blood. These include:

- **Bilirubin tests:** A high level of unconjugated bilirubin compared to levels of conjugated bilirubin suggest hemolytic jaundice.
- **Full blood count (FBC), or complete blood count (CBC):** This measures levels of red blood cells, white blood cells, and platelets.
- **Hepatitis A, B, and C tests:** This tests for a range of liver infections.

The doctor will examine the structure of the liver if they suspect an obstruction. In these cases, they will use imaging tests, including MRI, CT, and ultrasound scans.

They may also carry out an endoscopic retrograde cholangiopancreatography (ERCP). This is a procedure combining endoscopy and X-ray imaging.

A liver biopsy can check for inflammation, cirrhosis, cancer, and fatty liver. This test involves inserting a needle into the liver to obtain a tissue sample. The sample is then examined under a microscope.

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UNIT – IV - ORGAN FUNCTION TEST - SBC3104

UNIT 4 ORGAN FUNCTION TEST

Liver function test - Icteric index, Vandenberg test, plasma protein changes, PT. Renal function test : Clearance test – Urea, Creatinine, Inulin, PAH test, Concentration and dilution test. Gastric function test : Collection of gastric contents, examination of gastric residuum, FTM, stimulation test, tubeless gastric analysis.

UNIT 4 ORGAN FUNCTION TEST

Accessibility, ease of collection, and relatively low cost give serum chemistries an integral initial role in medical diagnosis. With the liver being a critical organ in the metabolism of carbohydrates, lipids and proteins as well as in first pass metabolism of exogenous medications, a great deal can be learned about hepatobiliary processes by having an appropriate grasp of specific liver chemistry tests. Liver enzymes are also commonly ordered for evaluation of other non-hepatic diagnoses and as part of health screening, which makes it imperative for all primary care physicians and specialists to have an accurate understanding of their normal values and an ability to interpret abnormal levels. Symptoms and signs of liver disease are often seen late in the disease. As a result laboratory testing helps in identifying and characterizing liver disease.

The term “liver function tests” is a misnomer, as many of these tests do not naturally reflect hepatobiliary function and are rather used as a determinant of liver injury. Furthermore, the origin of these tests may not be specific to the liver and, as such, abnormal results may be related to alternative organ injury. It is vital to use these test results in the context of patient history and physical examination in order to form an accurate diagnosis. For the purposes of this article, the liver chemistries that will be focused on will be bilirubin, alkaline phosphatase, aminotransferases and gamma-glutamyl transferase. In addition, albumin and prothrombin time will be discussed briefly. It will also provide a reference of normal laboratory values for an average adult male of individual tests based on information provided by the Mayo Clinic. Furthermore, as clinicians will be ordering these tests on a routine basis, the cost is relevant and Medicare pricing guidelines will be provided for each laboratory test. Following discussion of these tests individually, this article will focus on different patterns of abnormalities that relate to different disease processes.

Bilirubin

Bilirubin is a product of the digestion of hemoglobin. During the catabolism of erythrocytes, an initial unconjugated or “indirect” form of bilirubin is released into the reticuloendothelial system. As unconjugated bilirubin is water insoluble, it binds with albumin and is transported to the liver. Unconjugated bilirubin exists in majority as a component of total bilirubin when compared to direct bilirubin. In the liver, the unconjugated bilirubin enters the hepatocyte and is conjugated with glucuronic acid by the enzyme UDP-glucuronyltransferase (UGT), rendering it water soluble.1 This conversion process, from the unconjugated form to “direct”, or conjugated, form allows bilirubin to be transported through the canalicular membrane, mix with other components of bile within the biliary tree, and flow into the duodenum.2,3,4 In the duodenum, part of the direct bilirubin is reabsorbed while the rest is converted to urobilinogen by intestinal flora and excreted in the urine and stool. In addition, there is also a delta bilirubin, which can also be referred to as biliprotein, which is produced

by reaction of conjugated bilirubin with albumin.5 It is important to note that the half-life of this product is about 17-20 days (the same as albumin) accounting for prolonged jaundice in patients recovering from hepatitis or obstruction.6 The total bilirubin, which is a measure of both direct and indirect forms, has a normal reference range of 0.1-1.0 mg/dL.

Historically, in order to determine the serum levels of the two types of bilirubin, laboratories utilized the technique developed through the van den Bergh diazo reaction,7 which was able to separate water soluble conjugated bilirubin from unconjugated bilirubin for individual measurement. The accuracy of the direct bilirubin levels increased as the total bilirubin rose. The direct, or conjugated, bilirubin reference range is 0.0-0.3 mg/dL in a normal individual and should be no more than 20% of the total bilirubin when the total bilirubin is elevated due to non-hepatic causes, such as hemolysis or congestive heart failure. The indirect or unconjugated bilirubin is obtained by subtracting the direct bilirubin level from the total bilirubin.

Identifying the subtype of bilirubin, which is elevated, allows for accurate diagnosis when analyzing bilirubin levels. Isolated elevation of unconjugated bilirubin occurs mainly secondary to increased bilirubin production, decreased hepatic uptake and decreased bilirubin conjugation. Elevated levels of unsuccessful erythrocyte production, hemolysis, or reabsorption of large hematomas may lead to increased unconjugated bilirubin levels. Fulminant Wilson's disease can cause isolated elevation in unconjugated bilirubin secondary to the release of copper in the blood resulting in cellular lysis 8 Unsuccessful erythrocyte production exists in the setting of rapid heme and hemoglobin turnover in the bone marrow due to premature destruction of red blood cells. There exists evidence that in these conditions there is also presence of erythroid hyperplasia of bone marrow, reticulocytosis, increased iron turnover with diminished red blood cell incorporation, and hemosiderosis of hepatic parenchymal cells and Kupffer cells. However, why this occurs in the bone marrow is not known.8

Isolated elevation of unconjugated bilirubin also may be due to genetically inadequate UGT production preventing conjugation in disease processes such as Gilbert's syndrome and Crigler-Najjar disease.9 Gilbert's syndrome is a commonly seen disorder, which is relatively benign. The hyperbilirubinemia in Gilbert's syndrome is exacerbated with fasting.10 Elevated conjugated bilirubin can be caused secondary to inherited or acquired conditions. Genetic disease processes such as Dubin-Johnson and Rotor syndrome cause an impaired hepatocellular secretion of bilirubin into the bile canaliculus causing elevated conjugated bilirubin.11,12 As the anatomy suggests, elevations in conjugated bilirubin can occur secondary to hepatocellular dysfunction and cholestatic processes, which impair bile, flow. It has been found that despite the loss of liver function in hepatocellular disease processes, such as cirrhosis, UGT is produced at an increased rate in the remaining functioning hepatocytes forming conjugated bilirubin, such that increase in total bilirubin may not occur until late in the course of disease.13,14 Cholestasis can occur either because of impaired secretion into a bile canaliculus or impaired transit through the biliary tree and into the duodenum. Some of the causes of intra hepatic cholestasis are drug toxicity, primary biliary cirrhosis, primary sclerosing cholangitis, viral hepatitis, cholestasis of pregnancy, benign postoperative cholestasis, infiltrative liver diseases, sepsis and total parenteral nutrition. Certain causes of extra hepatic cholestasis are choledolithiasis,

malignant obstruction secondary to a mass in the pancreas, bile duct, gall bladder or ampulla, primary sclerosing cholangitis with an extra hepatic bile duct stricture, chronic pancreatitis and AIDS cholangiopathy.

Alkaline Phosphatase and gamma-glutamyl transferase

Alkaline phosphatase (ALP) is a zinc metalloenzyme and can be found in many different tissues, with most clinical relevance due to production in the bone, intestine, kidney or liver, and with more than 80% of serum ALP originating from bone or liver.15 The average serum level of ALP in a normal adult male is 45 to 115 U/L. There are certain physiological causes that lead to increased alkaline phosphatase, examples being the during the third trimester of pregnancy secondary to the influx of alkaline phosphatate from the placenta, in adolescents secondary to increase in bone turnover, or some individuals with an increased production of intestinal alkaline phosphatase which is familial and benign.10 Although it is generally ordered as part of routine liver chemistry, ALP abnormalities should be evaluated within the framework of hepatobiliary vs non-hepatobiliary diseases. In the liver, ALP is present in the hepatocytes on the cannalicular membrane, but is localized to the microvilli of the bile canaliculus, and elevated levels typically reflect a cholestatic disease process. The half life of ALP is one week and, as a result, even after the cholestatic process has resolved, the normalization of the ALP level may lag. In order to distinguish whether an isolated elevation of ALP is of hepatic origin, one could order ALP isozymes, which fractionate the total ALP into its tissues of origin. Alternatively, confirmation via a gamma-glutamyltransferase (GGT) level can be performed since GGT is more concentrated in hepatic tissue1 and is not present in bone. A concurrent elevation of ALP and GGT excludes a boney origin of the enzyme. It is important to note that initially the only notable abnormality that may be seen in infiltrative diseases such as primary biliary cirrhosis, sarcoidosis, primary sclerosing cholangitis, etc. is isolated elevations in ALP.1 Elevation in ALP is typically seen for duration of more than six months in these conditions. These cases normally require follow up with imaging or liver biopsy. ALP can also interestingly be raised in various neoplasms, which do not involve the bone or liver directly. This occurs secondary to an isozyme of ALP called the ‘Regan isoenzyme’.17

GGT is an enzyme primarily located in hepatocytes, epithelial lining of biliary ducts, pancreas, renal tubules and the intestine. The normal GGT level in adult male ranges from 9 to 48 U/L. GGT levels may be elevated in a large variety of common diseases such as diabetes, hyperthyroidism, pancreatitis, alcoholism, COPD and rheumatoid arthritis and also as a result of various medications like coumadin, carbamazepine, phenytoin, and barbiturates. Hence the specificity for liver disease is poor. Isolated GGT elevation may be seen in alcohol abuse. Note, however, the degree of GGT elevation does not directly correlate with the amount of alcohol consumed.11

Aminotransferases – Alanine aminotransferase (ALT) & Aspartate aminotransferase (AST)

In 1955, serum AST and ALT elevations were first noted in patients with known viral hepatitis and other hepatic specific diseases.18 Aminotransferases are so named as their enzymatic function is to transfer amino groups to form pyruvate via AST and form oxaloacetate via ALT. While present in several tissues including skeletal and cardiac muscle and erythrocytes, clinically relevant elevations are usually reflective of

liver disease, especially with respect to ALT elevations, isolated elevations of which should be assumed to reflect liver disease until proven otherwise. Their location within the hepatocyte is imperative to understanding the elevation patterns seen in various liver diseases. AST has two isoenzyme forms, with 80% operating as a mitochondrial isoenzyme; however, most of the circulating serum AST is derived from the cytoplasmic isoenzyme.19 Conversely, ALT is found only in the cytosol and is more specific to liver tissue. This makes an elevated ALT more specific for hepatocellular injury than AST. Elevated ALT levels however have also been noted in myopathic diseases.20

When determining a laboratory range for aminotransferases, important characteristics must be considered. Interestingly, as body mass index (BMI) increases, so does ALT; ALT is also higher in males relative to females. It is worth noting as well that AST levels may be 215% higher in African-American males.21 As ALT has more specificity for hepatocellular injury, cutoff values are important to ensure proper inclusion of patients with liver disease and elevated aminotransferases without unnecessary evaluation of patients with potentially normal levels.22 For the purposes of this article, we use a reference range for ALT as 7 to 55 U/L and AST as 8 to 48 U/L, with an understanding that a wide upper limit variability exists across different laboratories likely related to different reference standards.23 The magnitude of transaminase elevation relative to the upper limit of normal may help to narrow down the differential diagnosis for the cause of hepatocellular injury. Specifically, aminotransferase levels that are 15x or more the upper limit of normal deserve to be considered separately from mild or moderate elevations.13 Also to further classify pathologies, it is important to consider the ratio of ALT to AST.

Albumin

Albumin is a plasma protein produced solely in the liver, with a half-life of three weeks.24 As a result, a decrease in the albumin level compared to normal (<3.5 g/L) signifies a liver disease which has been occurring for greater than three weeks. Albumin level can be influenced by other factors such as the nutritional status, catabolism, hormonal factors, and urinary and gastrointestinal losses. As a result, these factors should be taken into consideration when interpreting albumin levels. In conclusion albumin is useful to interpret chronic and progressive liver disease and is also used to predict the prognosis of liver disease.

Prothrombin Time (PT)

All coagulation factors are produced in the liver. Factor VIII is produced in endothelial cells outside the liver in addition to being produced by the sinusoidal cells in the liver. The rate of conversion of prothrombin to thrombin requiring factors II, V, VII, X and fibrinogen is the measurement of prothrombin time (PT), thus a function of the liver. Prothrombin time can be prolonged even in a severe liver disease of < 24 hours secondary to the half life of most factors being equal to or less than 24 hours.2 It should also be noted that vitamin K is required in the production of factors II, VII IX and X. As a result, vitamin K deficiency can also cause prolonged prothrombin time. Some other factors that should be considered in cases of prolonged prothrombin time are warfarin therapy, disseminated intravascular coagulation (DIC), hypothermia and steatorrhea.

International Normalized Ratio (INR)

In order to avoid variability in laboratory values, international normalized ratio (INR) is more commonly tested instead of or in place of PT. The results are interpreted in the same way as PT would be interpreted. It is calculated according to a formula as follows: International normalized ratio = [patient PT/mean control PT] ISI (ISI = international sensitivity index).

Patterns Of The Liver Function Tests

Once a general understanding of each individual liver enzyme has been achieved, clinicians can then use the liver enzyme panel to begin recognizing patterns. Each test is important to understand; however the elevation of each in relation to the other parts of the panel is what is most useful in interpreting disease processes. In this section we will describe the different liver enzyme patterns and their associated disease processes.

The liver enzyme panel abnormalities can be broken down into two main subgroups, which will be discussed individually. These subgroups are a cholestatic pattern and a hepatocellular pattern. These subgroups will then be broken down further into respective categories. The R ratio has been described to assess whether the pattern of liver injury is hepatocellular, cholestatic, or mixed and may be applied in drug-induced liver injury.26 The R ratio is calculated by the formula R = (ALT value ÷ ALT ULN) ÷ (alkaline phosphatase value ÷ alkaline phosphatase ULN). An R ratio of >5 is defined as hepatocellular, <2 is cholestatic, and 2-5 is a mixed pattern. This paper will describe hepatocellar and cholestatic patterns.

Hepatocellular Disease Pattern

Hepatocellular pattern is diagnosed with a disproportionate elevation in AST and ALT relative to alkaline phosphatase. For the purpose of this paper we will use the following definitions to describe the magnitude of elevations of AST and ALT (Table 1).

It is important to identify acute liver failure or fulminant liver failure as diagnosed by hepatic encephalopathy and coagulopathy in a patient with no prior history of liver disease. For acute liver failure, it is not imperative to describe the magnitude of rise in ALT or AST. Rapid involvement of the consultancy groups and evaluation of liver transplant should be begun early on.

Causes of Aminotransferase Elevation Massive Elevation

(More than 10,000 times the upper limit) There is an overlap for the causes of elevation in AST and ALT between the groups of severe and massive elevation in AST and ALT. Ischemic liver disease, toxin and viruses related injuries can cause a massive elevation in AST and ALT. They are described further in the section below. It is also important to note that massive AST elevations can be seen in heat stroke and rhabdomyolysis.

Severe (15 times or greater than the upper limit of normal)

The severe elevations of serum aminotransferase levels are mainly found in the setting of excessive hepatocellular injury or necrosis in an acute setting. Although highly

elevated aminotransferases can suggest an acute injury, the actual quantification of hepatocyte necrosis cannot be inferred. Furthermore, extremely elevated aminotransferases do not indicate prognosis.10 The differential is limited and generally includes a drug or toxin induced hepatotoxicity, acute viral hepatitis, or ischemic hepatitis. Toxin-related hepatitis and acute viral hepatitis can increase the AST and ALT levels to >25 times the upper limit of normal, while ischemic hepatopathy can increase the levels to >50 times.

Many medications and toxins can cause liver injury. Some of the commonly seen medications are non-steroidal anti-inflammatory drugs, antibiotics, statins, antiepileptic drugs, and antituberculous drugs. It is also pertinent to note that certain herbal remedies and illicit drugs can cause liver injury.10 In the United States (USA), the leading cause of acute liver failure is acetaminophen poisoning, accounting for 46% of cases.27 Hepatotoxicity occurs when sulfate and glucuronide metabolic pathways become saturated, pushing more acetaminophen metabolism towards the cytochrome P450 pathway that results in the formation of the toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQ1). Chronic alcohol abusers can be more prone to acute liver failure in the setting of acetaminophen use and caution should be taken when treating these patients.28 Cytochrome P-450, principally cytochrome CYP2E1, metabolizes acetaminophen into a toxic metabolite, which is detoxified by glutathione under normal circumstances. CYP2E1 also detoxifies ethanol. Thus in chronic alcohol abusers, there is increase in CYP2E1 which increases the metabolism of acetaminophen into its toxic metabolites.29 Careful attention to occupational history should be given to patients with excessive aminotransferase elevation. Occupations that could lead to aminotransferase elevation include mushroom picking (Amanita phalloides) and those involved in the chemical industry (vinyl chloride).30

Acute hepatitis can also be caused by infection with any of the primary hepatitis viruses (A-E). Hepatitis B and hepatitis C are most prevalent in the USA, with hepatitis B being the leading cause of acute viral hepatitis in the USA. Viral serological tests are important to differentiate acute from chronic hepatitis. Hepatitis A is transmitted by the fecal oral route. It is a RNA virus, which has an

incubation period of a few weeks. The IgM antibodies to hepatitis A remain in the body for a period of three to six months after the infection.31

Hepatitis B is mainly spread through unsafe sexual practice, parental drug use or vertical transmission. Hepatitis B surface antigen (HBSAg) is positive in either acute or chronic hepatitis B infection, while HBV core IgM antibody generally specifies the acute state. Checking for HBVsAg and HBVDNA would indicate whether there is an active infection and infectivity of the virus. In addition, checking for hepatitis B surface antibody would indicate immunity to hepatitis B either secondary to resolution of a prior infection or vaccination.

Hepatitis C is transmitted through parental drug use, cocaine inhalation, blood transfusion prior to 1992, tattoos or body piercings, needle stick injury and unsafe sexual practices. Hepatitis C antibody testing is sensitive. Presence of HCV viremia should be confirmed in the setting of a positive antibody with the HCV RNA PCR assay, which has high sensitivity and specificity. Hepatology should be consulted for

patients positive for hepatitis C for evaluation of treatment, education on hepatitis C, and screening for cirrhosis and hepatocellular carcinoma.

Hepatitis D is an RNA virus that is only seen in the presence of hepatitis B surface antigen positivity. Suspicion for hepatitis D should arise when hepatitis B presents with fulminant hepatitis. Acute co-infection with hepatitis D is diagnosed when HBSAg, IgM anti-HBc, and total anti- HDV are present.

Another cause of acute hepatitis is hepatitis E virus. It is an enterically transmitted RNA virus. Another method of transmission of hepatitis E is through vertical transmission. Anti-HEV immunoglobulin IgM and IgG are used to detect hepatitis E. HEV RNA is used to confirm the presence of hepatitis E.

Occasionally, in the setting of acute hepatitis with excessively elevated AST and ALT levels, history and serology may not uncover a toxic or viral cause, and in these cases ischemic hepatitis should be considered. In ischemic hepatitis, the AST and ALT levels have the potential to increase to >50x the upper limit of normal. Several mechanisms can result in massive AST and ALT elevation, including decreased blood flow in instances such as hypotension, sepsis, hemorrhage, and myocardial infarctions.13 Concurrent elevation of lactate dehydrogenase (LDH) may suggest the diagnosis of ischemic hepatitis.32 These examples highlight the importance of a thorough history and physical examination to help stratify differential diagnoses in the setting of severe aminotransferase elevation.

Mild to Moderate (5-15 times the upper limit of normal)

Borderline and mild elevation in AST and ALT are seen in a variety of diseases. Moderate increase in AST and ALT often coincides with causes of mild and severe elevations. The two most commonly identified non-viral entities, alcoholic liver disease and non-alcoholic fatty liver disease, will be briefly described below.

Alcoholic Liver Disease

Alcohol ingestion can cause elevation in liver chemistries. Alcohol ingestion can be an independendant cause or can attenuate transaminitis concurrent with other chronic liver diseases. Alcohol can cause a wide spectrum of liver disease from fatty liver to alcoholic hepatitis to alcoholic cirrhosis. These conditions can also be present all at once in an individual. Liver biopsy is useful to identify the stage and severity of liver disease since the liver chemistries do not always correlate with these.33,34 The definition of significant alcohol consumption has been suggested as >210 g of alcohol per week in men and >140 g per week in women.35 In practice, an AST:ALT ratio of 2-3:1 raises the suspicion for alcoholic liver disease. It has been demonstrated that alcohol consumption leads to decrease in plasma pyridoxal 5′-phosphate.36 This decrease in levels results in a decrease in ALT activity. The decrease in plasma pyridoxal 5′-phosphate does not have an effect on AST leading to the ratio of AST:ALT being 3:1. Once alcohol abstinence is observed with appropriate nutritional uptake, plasma pyridoxal 5′-phosphate normalizes causing a normal ALT level.36,37,38 When alcohol use is felt to cause liver disease, it is strongly recommended to quit alcohol use, and appropriate counseling should be given.

Non-Alcoholic Fatty Liver Disease and Nonalcoholic Steato-Hepatitis

Nonalcoholic fatty liver disease (NAFLD) is defined as (a) there is evidence of hepatic steatosis, either by imaging or by histology and (b) absence of causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of medication that could cause fatty liver injury, or hereditary disorders. NAFLD is commonly seen in individuals with the metabolic syndrome, characterized by obesity, diabetes mellitus, and dyslipidemia. Histologically, NAFLD can be characterized as non-alcoholic liver (NAFL) or non alcoholic steato-hepatitis (NASH). Differentiation of NAFL from NASH is characterized by the presence of inflammation and hepatocellular injury, in the form of ballooning of the hepatocytes, with or without fibrosis, in the setting of NASH. It is concluded that patients with NAFL have a rather benign, slow progression (if any) histologically, while NASH can rapidly progress to the cirrhotic stage.39,40 Steatohepatitis and fibrosis as seen in NAFLD cannot be assessed accurately with serum transaminases, emphasizing the importance of further evaluation with imaging studies such as ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) and magnetic resonance elastrography (MRE) or with liver biopsy. MRE has proven to be a non-invasive, feasible and accurate modality to identify hepatic steatosis and fibrosis. MRE quantifies the extent of hepatic fibrosis with great accuracy. As compared to ultrasound, MRE is beneficial secondary to being non-technician dependant and being able to identify small amount of fibrosis. However liver biopsy continues to be the preferred modality to differentiate NAFL and NASH. The procedure related morbidity and mortality, cost and sampling error of liver biopsy has lead to interest is identifying non invasive biomarkers to identify steatohepatitis and fibrosis in NAFLD. The NAFLD fibrosis score, enhanced liver fibrosis (ELF) panel and transient elastography are identified as non-invasive methods to identify the spectrum and stage of NAFLD. The NAFLD fibrosis score is comprised of six variables (age, BMI, hyperglycemia, platelet count, albumin, AST/ALT ratio) and it is calculated using the published formula (http://nafldscore.com). Cytokeratin-18 (CK18) fragments have been investigated extensively as novel biomarkers for the presence of steatohepatitis in patients with NAFLD.41,42 Weight loss in the form of decreased caloric intake and exercise is recommended as the primary treatment. Vitamin E (a-tocopherol) administered at daily doses of 800 IU/day improves liver histology in many non-diabetic adults with biopsy-proven NASH and therefore it should be considered as a first-line pharmacotherapy for this patient population.

AST/ALT Ratio

AST/ALT ratios are of great diagnostic aid. An AST:ALT ratio of 2-3:1 raises the suspicion for alcoholic liver disease as discussed previously under the section of alcoholic liver disease. ALT has a longer half life compared to AST. The half life of ALT is 47 +/- 10 hours and that of AST is 17+/- 5 hours. In cholecystitis secondary to gallstone impaction in the distal cystic duct or choledocholithiasis, there is as increase in the AST:ALT ratio initially. However once disimpaction of the stone either spontaneously or iatrogenically is achieved, there is a reversal of this ratio secondary to ALT having a longer half life as compared to AST. It is also important to note that in chronic hepatitis the AST:ALT ratio may be increased up to 1. In advanced hepatic fibrosis, there is a reversal in the AST:ALT ratio in chronic as compared to acute hepatitis. Studies have shown that this is mainly caused by to the increased catabolism of ALT. Earlier it was thought to be secondary to increased production of AST and

decreased production of ALT, which has now proven to not be the cause of the ratio reversal.45,46

Cholestatic Liver Disease Pattern

Cholestatic injury is defined as disproportionate elevation in alkaline phosphatase level as compared with AST and ALT levels. Anatomic obstructions to bile flow (extrahepatic cholestasis) or inability to form bile by the hepatocytes (intra-hepatic cholestasis) can cause a cholestatic injury pattern.

Once the origin of alkaline phosphatase has been identified as the liver, it is recommended to evaluate with an ultrasound or other form of liver imaging to identify whether the source is intra-hepatic or extra-hepatic. An MRI/MRCP (magnetic resonance cholangiopancreatography), endoscopic retrograde cholangiopancreatography and/or endoscopic ultrasound can be ordered to better examine the bile duct morphology. In the presence of biliary dilation, the source of a cholestatic pattern is most likely extra hepatic, while the absence would indicate an intra hepatic source. Causes of cholestatic liver disease as outlined in Figure 1.

For intrahepatic cholestasis, autoimmune markers including antimitochondrial antibody, antinuclear antibody, and smooth muscle antibody should be checked to assess for PBC or auto- immune cholangiopathy. Finally, pregnancy testing in women of childbearing age should be done to assess for intrahepatic cholestasis of pregnancy. Other infiltrative disorders may raise the alkaline phosphatase and cause intrahepatic cholestasis, including sarcoidosis, atypical fungal infection, or malignancies. In these instances of infiltrative diseases, a liver biopsy may be considered to assess for primary biliary cirrhosis or other infiltrative diseases.

The following points highlight the nine main tests for assessing the functions of liver. The tests are: 1. Bile Pigment Metabolism in Health and in Jaundice 2. Tests for Carbohydrate Metabolism 3. Tests for Plasma Protein Concentration 4. Tests for Detoxifying Functions 5. Test for Excretion of Foreign Substances 6. Tests for Blood Coagulation 7. Tests for Serum Enzymes and Others.

Tests for Assessing the Functions of Liver:

1. Bile Pigment Metabolism in Health and in Jaundice
2. Tests for Carbohydrate Metabolism
3. Tests for Plasma Protein Concentration
4. Tests for Detoxifying Functions
5. Test for Excretion of Foreign Substances
6. Tests for Blood Coagulation
7. Tests for Serum Enzymes
8. Test for the Conversion of Ammonia to Urea
9. Glutamine Content of Cerebrospinal Fluid

Test # 1. Bile Pigment Metabolism in Health and in Jaundice:

When the red blood cells have lived out their life span (averaging 120 days), their cell membranes rupture and the released hemoglobin is phagocytized by the reticuloendothelial cells throughout the body. The hemoglobin is first split to heme

and globin, and the heme ring is opened to give a straight chain of four pyrrole nuclei from which bile pigments are formed.

The first pigment formed is biliverdin which is rapidly reduced to free bilirubin. This free bilirubin immediately combines very strongly with the plasma albumin and is transported throughout the blood and interstitial fluids.

Even when bound with the plasma protein, this bilirubin is still called “free bilirubin”. 80 per cent of the free bilirubin then conjugates in the liver with glucuronic acid to form bilirubin glucuronide; 10 per cent conjugates with sulphate to form bilirubin sulphate and the rest 10 per cent conjugates with a multiple of other substances.

This conjugated bilirubin is excreted by an active transport process into the bile canaliculi. A small portion of the conjugated bilirubin formed by the hepatic cells returns to the plasma either directly or indirectly by absorption. Therefore, a small portion of the conjugated bilirubin is always available in the extracellular fluid.

Bilirubin is then converted by bacterial action into mesobilirubin which is reduced to mesobilirubinogen in the intestine. This compound is further reduced to stercobilinogen (Urobilinogen) which is highly soluble. Some of the urobilinogen is reabsorbed through the intestinal mucosa into the blood.

Most of this is re-excreted by the liver back into the gut; but about 5 per cent of it is excreted by the kidneys into the urine. After exposure to air in the urine, the urobilinogen becomes oxidized to urobilin. The stercobilinogen in the feces becomes oxidized to stercobilin.

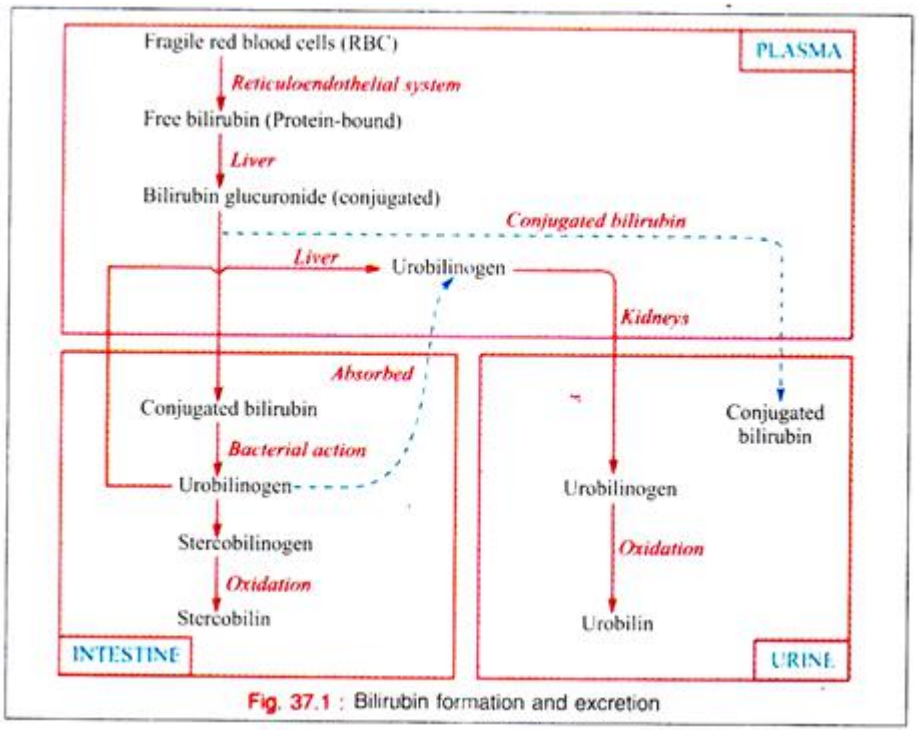


Fig. 37.1 : Bilirubin formation and excretion

Jaundice:

“Jaundice” means a yellowish tint to the body tissues, including yellowness of the skin and also of the deep tissues. The usual cause of jaundice is the increased bilirubin content in the extracellular fluids, either free or conjugated bilirubin.

The normal plasma concentration of bilirubin (both free and conjugated forms) averages 0.5 mg per 100 ml of plasma. But in certain abnormal conditions this can rise to as high as 40 mg per 100 ml. The skin becomes yellow when the concentration rises to about three times the normal.

Causes:

a. Increased destruction of red blood cells with rapid release of bilirubin into the blood.

b. Excessive production of bilirubin beyond the capacity of normal liver to excrete it.

c. Dysfunction of liver cells resulting in failure to convert bilirubin into bilirubin glucuronide form in the liver and excrete it in the bile.

d. Obstruction of the bi le ducts to make usual flow of the bile to the duodenum.

Classification:

Rolleston and McNee classified jaundice as follows:

a. Hemolytic jaundice.

b. Obstructive jaundice.

c. Hepatic jaundice.

Rich classified jaundice as follows:

a. Retention jaundice (overproduction of unconjugated bilirubin).

b. Regurgitation jaundice (due to necrosis of liver cells and obstruction of bile ducts).

A. Hemolytic jaundice:

a. The excretory function of the liver is not at all impaired.

b. The red blood cells are hemolyzed rapidly by hemolytic anemia’s by the action of some drugs, by malarial or viral infections, and by incompatible blood transfusion. The hepatic cells simply cannot excrete the bilirubin as rapidly as it is formed. Therefore, the plasma concentration of free bilirubin rises markedly.

c. The rate of formation of urobilinogen in the intestine is greatly increased and much of this is absorbed into the blood and later excreted in the urine. The colour of the feces becomes dark brown.

B. Obstructive Jaundice:

a. This happens due to the obstruction in the bile duct preventing the flow of bile into the intestine. The obstruction is caused by the blocking of the bile passage by gallstones, by enlarged glands due to tumour of the head of the pancreas, and by stricture of narrowing of the bile duct as a result of surgery.

b. The rate of bilirubin formation is normal; but the bilirubin cannot pass from the blood into the intestines. The free bilirubin then enters the liver cells and becomes conjugated in the usual way.

c. The conjugated bilirubin is then returned to the blood probably by rupture of the congested bile canaliculi and direct emptying of the bile into the lymph leaving the liver.

d. The excess bilirubin is excreted in the urine producing a deep yellow or brownish colour. The stools become clay coloured and bulky containing excessive amount of fat. The serum alkaline phosphatase concentration is usually high.

C. Hepatic jaundice (Toxic and infective jaundice):

a. Hepatic jaundice is caused by infection, toxins and liver poisons. Infection with virus is the most common cause. The infective organisms cause damage to the liver parenchymal cells.

b. The conjugation of bilirubin in the liver is thereby affected. Hence, both free and conjugated bilirubin concentration is increased in the serum.

c. The urine becomes highly coloured due to the presence of conjugated bilirubin and urobilinogen.

(i) Van den Berg reaction to differentiate between hemolytic and obstructive jaundice diagnostically:

If a freshly prepared diazotized sulphanilic acid reagent is added to serum, conjugated bilirubin gives a reddish violet colour within a minute known as the "direct" Van den Berg reaction. The free bilirubin (unconjugated bilirubin) of the serum does not develop any colour within one minute but the colour is formed if alcohol is added to the mixture.

Alcohol precipitates the protein and makes free the free bilirubin from its protein complex so that it can then combine with the Van den Berg reagent. This result is called the "indirect" Van den Berg reaction. Therefore, in hemolytic jaundice an indirect Van den Berg reaction occurs (increased free bilirubin) and in obstructive jaundice a direct Van den Berg reaction takes place (increased conjugated bilirubin).

If a faint pink colour is formed after one minute and deepening of the colour results in 2 or 3 minutes in some cases, that indicates clearly that both conjugated and free bilirubin's are present in the serum. This reaction is said to be "biphasic" reaction.

In the total obstruction of bile flow, no bilirubin can reach the intestines to be converted into urobilinogen by bacteria. Therefore, urobilinogen is not reabsorbed into the blood and is not excreted by the kidneys into the urine. So in total obstructive jaundice, tests for urobilinogen in the urine are completely negative.

The stool becomes clay coloured for lack of stercobilin, but not free bilirubin. Therefore, in severe obstructive jaundice, large quantities of conjugated bilirubin appear in the urine. This can be known by shaking the urine and observing the foam, which becomes intense yellow in colour.

(ii) Biochemical tests for jaundice:

The biochemical tests are the following:

a. Serum bilirubin concentration and nature of Van den Berg reaction.

b. Serum alkaline phosphatase activity and SGPT activity.

c. Urine test for urobilinogen and bilirubin.

d. Thymol flocculation test and colloidal gold test.

e. Feces colour.

The biochemical findings in three types of jaundice are given in table 37.1.

Table 37.1 : Biochemical changes in jaundice

No.	Tests	Hemolytic	Obstructive	Hepatic
1.	Serum bilirubin	2 to 3 mg/100 ml (free bilirubin)	Up to 50 mg/100 ml (conjugated bilirubin)	Up to 20 mg/100 ml (both types of bilirubin)
2.	Van den Berg reaction	Indirect	Direct	Biphasic
3.	Test for bile pigments in urine	Urobilinogen (Schlesinger's test positive)	Conjugated bilirubin (Fouchet's test and Gmelin's test positive)	Urobilinogen and conjugated bilirubin
4.	Feces colour	Dark brown	Clay colour	Varies
5.	SGPT activity	5 to 30 units (normal)	35 to 100 units (Raised slightly)	100 to 300 units (Raised markedly)
6.	Serum alkaline phosphatase activity	3 to 12 K.A. units and up to 25 in children (normal)	30 to 100 K.A. units (Raised markedly)	15 to 30 K.A. units (Raised slightly)
7.	Serum 5-nucleotidase activity	Negative	Raised markedly	Raised slightly
8.	Thymol flocculation test	Negative	Negative	Positive
9.	Colloidal gold test	Negative	Negative	Positive

(iii) Icteric Index:

The icteric index shows the degree of jaundice by measuring the intensity of yellow colour of the serum. Serum is diluted with normal saline until it matches the colour of 1 in 10,000 solution of potassium dichromate. The dilution factor is termed Icteric Index. The Icteric Index in normal person is 4 to 6 units. In latent jaundice, it is 3 to 14 units. In clinical jaundice, it is higher than 15 units. Carotene present in serum interferes its determination.

(iv) Urine Bilirubin:

The urobilinogen in urine can be detected by Schlesinger's test. The concentration of urobilinogen is more in hemolytic and hepatic jaundice. Fouchet's and Gmelin's test are both positive in obstructive jaundice. These tests indicate the presence of conjugated bilirubin in urine in obstructive jaundice.

(v) Bile Pigments in Feces:

The quantity of stercobilinogen depends on the quantity of bilirubin entering into the intestine. Stercobilinogen content of feces of patients with stone obstruction is nearer the normal level (10 to 150 mg/ day in adults) while it is low or absent (0 to 5 mg/day) in those with malignant obstruction.

(vi) Congenital Hyperbilirubinemia:

(a) Gilbert's syndrome:

In this condition, there is defective intracellular transport of bilirubin. In some cases, there is defective hepatic conversion of bilirubin to bilirubin diglucuronide. Therefore, the serum-free bilirubin concentration is high ranging from 4 to 6 mg/100 ml, sometimes it may rise to 12 mg 100 ml; The serum shows indirect Van den Berg reaction.

(b) Lucey-Driscoll syndrome:

This condition appears in newborn infants. The serum bilirubin concentration is very high (up to 60 mg/100 ml). This syndrome occurs due to the presence of a substance (probably steroid) which inhibits the conversion of bilirubin into bilirubin

diglucuronide in the liver. This inhibitor disappears one month after the birth of the infants having this syndrome.

(c) Dubin-Johnson syndrome:

In this condition, there is defective excretion of conjugated bilirubin by liver cells into bile. Conjugated bilirubin is found in urine. Alkaline phosphatase level of serum is normal.

(d) Crigler-Najjar syndrome:

This syndrome appears in new born infants. The serum bilirubin level rises to 20 mg/100 ml or more within a few days after birth. This condition is a familial incidence. Bilirubin is not converted to bilirubin diglucuronide due to the deficiency of the enzyme glucuronyl transferase in the liver.

The bile does not contain conjugated bilirubin. The serum bilirubin level comes down to normal level in those infants who survive.

Test # 2. Tests for Carbohydrate Metabolism:

(i) Galactose Tolerance Test:

This test is done in the morning after overnight fast. Fasting blood sample is taken. The individual is then given to ingest a galactose solution containing 40 grams of galactose in 300 ml of water. Blood is drawn at half an hour interval for 2 hours. Galactose content of the blood samples are then determined after removing the glucose by fermenting with yeast.

The normal blood galactose level is 0 to 160 mg/100 ml. In infective and toxic hepatitis, values may go up to 500 mg/100 ml of blood. In cirrhosis of the liver, values up to 500 mg/100 ml of blood are also found depending on the severity of the disease.

(ii) Fructose Tolerance Test:

This test is also performed in the morning after overnight fast. The individual is administered 50 grams of fructose dissolved in 300 ml of water. Fasting blood sample is taken. Blood is also taken at half an hour intervals for 2 hours after fructose ingestion. The total blood sugar (glucose + fructose) is estimated.

In normal subjects, the highest blood sugar level does not exceed the fasting level by more than 30 mg/100 ml. Blood sugar levels up to 150 mg/100 ml are found in patients with infective hepatitis. This test is less sensitive than the galactose tolerance test.

Test # 3. Tests for Plasma Protein Concentration:

Albumin, fibrinogen, and some of the α- and β- globulins are synthesized in the liver. In advanced liver diseases, the albumin content is decreased and globulin content increased.

Edema may develop when the plasma albumin level falls below 2.5 per cent. The globulin content may increase up to 5 per cent in some cases. Fibrinogen values in normal persons range from 0.2 to 1.03 per cent and it may fall to 0.1 gm. in severe liver disorder, such as acute hepatic necrosis.

(i) Electrophoretic Separation of Plasma Proteins:

The percentage of different proteins determined by paper electrophoresis in normal subjects are as follows:

Albumin	55.2%
Globulin	44.8%
Albumin/Globulin ratio	1.23%
α_1-Globulin	5.3%
α_2-Globulin	8.7%
β-Globulin	13.4%
γ-Globulin	11.0%
Fibrinogen	6.5%

The flowing results are obtained in liver diseases:

Chronic infective hepatitis	.....	γ-globulin increased
Cirrhosis of the liver	.....	Albumin content decreased
Necrosis of the liver	.....	Fibrinogen content decreased

(ii) Flocculation Tests:

(a) Thymol turbidity test:

The degree of turbidity is measured against standards containing 10, 20, 30, 40, . . . 100 mg per 100 ml of protein when serum is mixed with a buffered solution of thymol. A turbidity equal to that of the 10 mg protein standard is taken as 1 unit by McLagan. In normal subjects, the thymol units range from 0 to 4 units. In infective hepatitis, the values range from 5 to 20 units. In obstructive jaundice, only 8 per cent give positive result. The thymol flocculation test will be positive in all cases in which the turbidity is positive.

(b) Serum colloidal gold test:

The results obtained in this test in subjects suffering from liver diseases are similar to those obtained with thymol turbidity test.

(c) Zinc sulphate test:

This test is positive in all cases of infective hepatitis and cirrhosis. In normal persons, serum y-globulin content is 2 to 8 units but the values rise from 15 to 80 units in infective hepatitis and cirrhosis.

Test # 4. Tests for Detoxifying Functions:

(a) Hippuric acid Synthesis Test:

The liver detoxicates benzoic acid by reacting it with glycine to form hippuric acid which is excreted in urine. The liver is able to synthesize sufficient glycine to conjugate with benzoic acid to form hippuric acid.

The test should begin at least 3 hours after a light breakfast. The patient empties the bladder and drinks sodium benzoate in about 200 ml of water. Urine is collected for a period of 4 hours from the time of ingestion of sodium benzoate. The amount of hippuric acid excreted is determined.

In normal persons, 60 per cent of the benzoic acid taken should be excreted as hippuric acid. The excreted should be 4.5 grams. Smaller quantities are excreted in acute or chronic liver damage.

Test # 5. Test for Excretion of Foreign Substances:

Bromsulphthalein (BSP) Test:

When bromsulphthalein dye is injected, it circulates in the blood in combination with albumin. Normal subjects after injection of 5 mg BSP per kg body weight retain less than 10 per cent of the dye in 30 minutes, and 7 per cent in 45 minutes.

At 60 minutes, no dye is retained. This is the most sensitive and dependable liver function test. It is particularly useful for evaluating suspicious or slightly positive results obtained by flocculation tests in the absence of hyperbilirubinemia.

If the liver function is impaired, the dye is excreted slowly and up to 50 per cent of the dye will be retained in the body at the end of 45 minutes after injection. The test is more useful in the diagnosis of liver cell damage without clinical jaundice, in chronic hepatitis and in cirrhosis of the liver.

Test # 6. Tests for Blood Coagulation:

Prothrombin Time Test:

Prothrombin (Factor II) and factors VII, IX and X involved in the coagulation of blood are synthesized in the liver in the presence of Vitamin K.

Deficiencies of these can occur for two reasons:

(i) In the presence of parenchymal cell damage, synthesis is impaired despite adequate supplies of Vitamin K.

(ii) In the absence of bile as in cholestasis and obstructive jaundice, the Vitamin K is not absorbed from the intestines and their synthesis is affected.

Shortening of prothrombin time after parental Vitamin K therapy suggests cholestasis; while lack of response to Vitamin K indicates liver damage

Test # 7. Tests for Serum Enzymes:

Certain enzymes are released from liver into the blood due to the damage to liver cells. The levels of SGOT, SGPT, LDH and isocitrate dehydrogenase are increased. The levels of these enzymes are increased in viral hepatitis and reach their maximum soon after the onset of jaundice, and then decrease slowly.

Very high levels occur in toxic hepatic necrosis. Levels in cirrhosis may be moderately raised but only if the process is active. Choline esterase levels are decreased in liver cell dysfunction.

Test # 8. Test for the Conversion of Ammonia to Urea:
The normal range of blood ammonia is 40 to 75 mg ammonia nitrogen per 100 ml. In cirrhosis of the liver, blood ammonia may be increased to over 250 mg/100 ml. High values are found in hepatic coma.

Test # 9. Glutamine Content of Cerebrospinal Fluid:
The normal range of glutamine in cerebrospinal fluid is from 6 to 14 mg per 100 ml. In cirrhosis of the liver, higher values ranging from 16 to 31 mg/ 100 ml have been reported. In hepatic coma, still higher values ranging from 30 to 54 mg/100 ml have been reported.

RENAL FUNCTION TESTS

Biochemical markers play an important role in accurate diagnosis and also for assessing risk and adopting therapy that improves clinical outcome. Over decades research and utilization of biomarkers has evolved substantially. National Institute of Health (NIH) 2001 defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological, pathologic processes, or pharmacologic responses to a therapeutic intervention. As markers of renal function creatinine, urea, uric acid and electrolytes are for routine analysis whereas several studies have confirmed and consolidated the usefulness of markers such as cystatin C, β-Trace Protein.

Creatinine

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass. Creatinine is a commonly used as measure of kidney function.The normal creatinine clearence test valve is 110-150ml/min in male and in female it is 100-130ml/min.The National Kidney Disease Education Program recommends calculating glomerular filtration rate from serum creatinine concentration. The creatinine clearance test is used to monitor the progression of renal disease. The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the “normal” interval. In chronic renal failure and uremia, an eventual reduction occurs in the excretion of creatinine by both the glomeruli and the tubules. Creatinine values may alter as its generation may not be simply a product of muscle mass but influenced by muscle function, muscle composition, activity, diet and health status. The increased tubular secretion of creatinine in some patients with kidney dysfunction could give false negative value. The elevated values are also seen in muscular dystrophy paralysis, anemia, leukemia and hyperthyroidism. The decreased values are noticed with glomerulonephritis, congestive heart failure, acute tubular necrosis, shock, polycystic kidney disease, and dehydration.

Urea

Urea is major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. In kidneys urea is filtered out of blood by glomerulli and is partially being reabsorbed with water. The most frequently determined clinical indices for estimating renal

function depends upon concentration of urea in the serum. It is useful in differential diagnosis of acute renal failure and pre renal condition where blood urea nitrogen–creatinine ratio is increased. Urea clearance is a poor indicator of glomerular filtration rate as its overproduction rate depends on several non renal factors, including diet and urea cycle enzymes. Increased blood urea nitrogen (BUN) is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract. The high BUN levels can sometimes occur during late pregnancy or result from eating large amounts of protein-rich foods. If the BUN level is higher than 100 mg/dL it points to severe kidney damage whereas decreased BUN is observed in fluid excess. Low levels are also seen in trauma, surgery, opioids, malnutrition, and anabolic steroid use.

Inulin

Fructose polymer inulin (MW 5kDa) satisfies the criteria as an ideal marker of glomerular filtration rate. Rapid measurement of glomerular filtration rate by an inulin single-bolus technique would be practically useful[21].

Iohexol

A new technique of measuring iohexol clearance using timed dried capillary blood spots was shown by Mafham M et al[22]. Blood spot iohexol clearance showed potential in estimating glomerular filtration rate accurately in large-scale epidemiological studies especially among individuals without established chronic kidney disease[22]. Plasma clearance after single injection of iohexol gives a good estimate of glomerular filtration rate and is advantageous for the patients and clinicians. Iohexol clearance is also used to estimate residual renal function in hemodialysis patients[23].

Radioactive Markers

In recent decade radioisotopes markers have been used to measure glomerular filtration rate. Some of them to mention are 125iodine (I)-iothalamate, 51CrEDTA ethylenediamine tetra acetic acid, 99mTc-DTPA (diethylene triamine penta acetic acid) and 99mTc mercapto acetyl triglycine. Renal 125iodine (I)-iothalamate clearance, is a simple and accurate test after a single subcutaneous injection, to measure glomerular filtration rate in adults[24]. Efficiency of 125 iodine (I)-iothalamate was shown by Geeta Bajaj et al. The same author found renal clearance of 125 iodine (I)-iothalamate was reproducible, simple, and practical in healthy children and those with mild and advanced renal disease. In one of the study the mean renal extraction of Cystatin C was equal to the mean renal extraction of 125 iodine (I)-iothalamate in hypertensive patients, suggesting tubular secretion of Cystatin C[25]. It was possible to get an accurate determination of 5lCr-EDTA clearance from a single-plasma sample in adults by applying the mean sojourn time-based approach previously shown to be very precise for determination of 99mTc-DTPA single-sample clearance[26]. 5lCr EDTA- glomerular filtration rate is suggested for systemic lupus erythematosus patient with suspected renal involvement even when the serum creatinine concentration and creatinine clearance are normal[27]. The limitation of this marker is that glomerular filtration rate measured by 5lCr EDTA can be overestimated in patients with severe oedema[26].

Concentration and Dilution methods

Serum osmolality was measured directly using osmometry, or estimated based on the direct measurement of the concentrations of the osmotically active substances (i.e. sodium, glucose, blood urea nitrogen, and ethanol). The difference between the measured osmolality and the calculated molarity is referred to as the osmole gap[36]. Laloë PA et al[37] observed severe hyponatraemia in some of the patients by measuring urine osmolality and urine sodium. According to Jeff MS[38] there are some genes which are involved in urine concentration which may encode solute-transport proteins and the vasopressin receptors. These molecular mechanisms show the reduction in urine-concentrating ability with aging that predicts various changes in kidney function. While Landon S et al[39] showed that aquaporin-1 has a physiologic role in renal function and is also essential for maximal urinary concentrating ability. In a complete deficiency of Aquaporin-1 there is defective urine concentrating ability.

Electrolyte

Electrolyte panel is frequently used to screen for an electrolyte or acid-base imbalance and to monitor the effect of treatment on a known imbalance that is affecting bodily organ function. The test for electrolytes includes the measurement of sodium, potassium, chloride, and bicarbonate for both diagnosis and management of renal, endocrine, acid-base, water balance, and many other conditions. Potassium used as a most convincing electrolyte marker of renal failure. The combination of decreased filtration and decreased secretion of potassium in distal tubule during renal failure cause increased plasma potassium. Hyperkalemia is the most significant and life-threatening complication of renal failure.

Tests for Determination of Gastric Function

The following points highlight the four main tests for determination of gastric function. The tests are: 1. Examination of Resting Contents 2. Fractional Gastric Analysis using Test Meals 3. Examination of Contents after Stimulation 4. Tubeless Gastric Analysis.

Tests for Determining Gastric Function:

1. Examination of Resting Contents
2. Fractional Gastric Analysis using Test Meals
3. Examination of Contents after Stimulation
4. Tubeless Gastric Analysis

Test # 1. Examination of Resting Contents:

After a night's fast the stomach contents are completely removed by passing the tube. The following characteristics are important in the diagnosis of diseases of stomach:

i. Volume:

(a) Only 20 to 50 ml of resting contents are obtained in normal cases.

(b) An increase in volume may be due to hyper-secretion of gastric juice, retention of gastric contents owing to delayed emptying of the stomach, and regurgitation of the duodenal contents.

ii. Consistency:

(a) The normal gastric juice is fluid in consistency and does not contain any food residue and may contain small amounts of mucus.

(b) Food residues are present in case of carcinoma of the stomach.

iii. Colour:

(a) In case of normal person, the gastric residue is clear or it may slightly yellow or green due to regurgitation of bile from duodenum.

(b) A dark red or brown colour may be observed due to the presence of altered blood or fresh-blood.

iv. Bile:

Increased quantities of bile shows abnormality which is a result of intestinal obstruction or ideal stasis.

v. Blood:

(a) Blood is not present in normal cases.

(b) Presence of small amount of fresh blood may be traumatic.

(c) Brown or reddish-brown blood may occur in gastric ulcer and sometimes in gastric carcinoma due to the formation of dark brown acid hematin as a result of the hemolysis of red blood cells by HCl.

(d) Bleeding may also occur from gastritis.

vi. Mucus:

(a) A small amount of mucus may be present in normal cases.

(b) Increased amount of mucus is present in gastritis and in gastric carcinoma. Presence of mucus is inversely proportional to the amount of HCl present.

(c) Swallowed saliva may contain excess of mucus.

vii. Free and Total Oddity:

(a) The acidity is determined by titration with a standard solution of NaOH using methyl orange or Topfer's reagent which indicates end point by the change of red to yellow colour or using phenolphthalein indicator which shows end point by the change of yellow to red colour.

(b) The presence of the amount of free HCl is free acidity; the complete titration shows the total acidity which is composed of protein hydrochloride and any organic acid; the difference between two titrations gives the combined acid.

(c) The result is expressed as ml of 0.1 N HCl per 100 ml of gastric contents. This is same as mEq/litre. This figure is obtained by multiplying the above titration by 10.

(d) The normal values of free acid is 0 to 30 mEq/L and that of total acid is 10 to 40 mEq/L.

viii. Organic Acids:

(a) The presence of large amounts of lactic acid and butyric acid in achlorhydria and hypochlorhydria indicates the remaining of residual foods in the stomach. In absence of HCl, the microorganisms ferment the food residues producing lactic acid and

butyric acid, (b) Achlorhydria is associated with retention of food residues and is found in carcinoma stomach.

Test # 2. Fractional Gastric Analysis using Test Meals:
This consists of:
i. Introduction of Ryle's tube in stomach of a fasting individual.
ii. Analysis of residual gastric contents after collection.
iii. Ingestion of test meal.
iv. Collection of 5 to 6 ml gastric contents after meal by aspiration using a syringe and analysis of the samples.
(i) Test Meals:
(a) Oatmeal is prepared by adding 2 tablespoonful's of oat meal to one quart of boiling water.
(b) "Ewald" test meal consists of two pieces (35 gm.) of toast and 250 ml light tea.
(c) Either of the meal is consumed by the patient and either of the tubes in introduced after one hour.

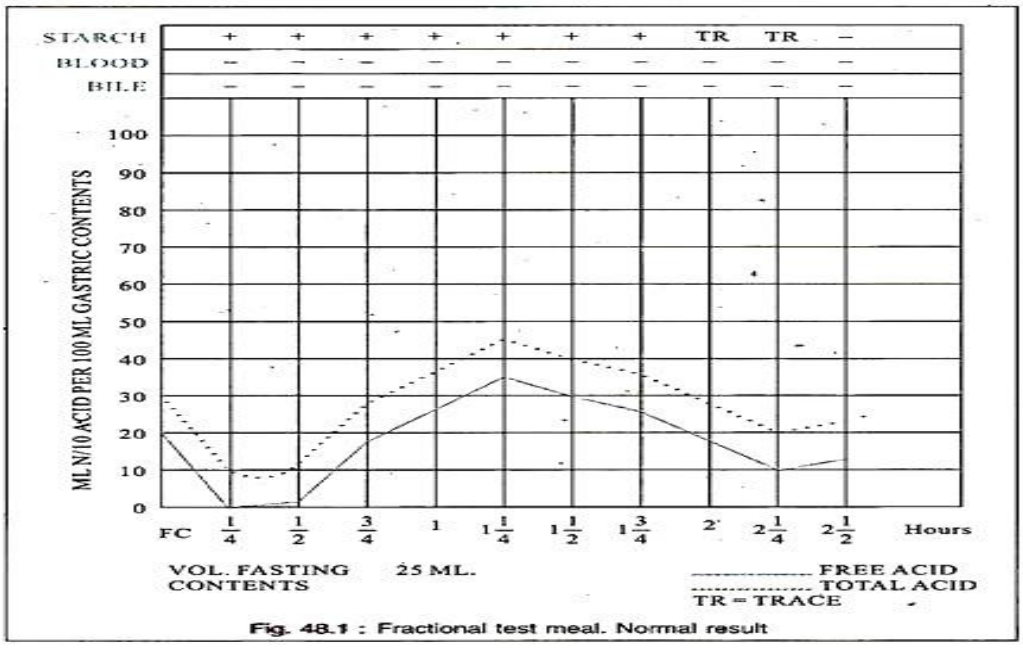


Fig. 48.1 : Fractional test meal. Normal result

(ii) Collection of Samples:
(a) About 10 ml gastric contents are collected at an interval of exactly 15 minutes by means of syringe attached to the tube.
(b) If the stomach is not empty at the end of 3 hours, the remaining stomach contents are removed and the volume is also noted.

(c) Each sample is strained through a fine cloth.
(d) The strained samples are analysed for free and total acidity and the residue on the cloth is examined for mucus, bile, blood, and starch.
(iii) Results and Interpretation:
(a) In normal health, after taking the meal, the free acid is found after 15 to 45 minutes (See figure below). The free acid then steadily rises to reach the maximum at about 15 minutes to 1/2 hour, after which the concentration of free acid begins to fall. The free acid ranges from 15 to 45 mEq/litre at the maximum with total acid at about 10 units higher. Blood is not present and appreciable amount of bile is also not present.
(b) In hyperchlorhydria, free acidity exceeds 45 mEq/litre but the combined acidity remains the same as in normal persons. Hyperacidity is found in duodenal ulcer in which a climbing type of curve is formed in gastric ulcer in which 50 per cent cases give normal results, and blood may be present, in gastric carcinoma in which small percentage show hyperacidity and blood, in jejunal and gastrojejunal ulcers in which there may be hyperacidity after operation.
(c) In hypochlorhydria, low acidities are found in carcinoma of stomach and in atonic dyspepsia. Free HCl is absent in gastric secretion in pernicious anemia.
(d) In achlorhydria, no HCl secretion but pepsin is present.
(e) In achylia gastrica, gastric secretion is completely absent due to advanced cases of cancer of stomach, advanced cases of gastritis, and acute pernicious anemia.

Test # 3. Examination of Contents after Stimulation:
A. Alcohol Stimulation:
(i) The Ryle's tube is passed into the stomach after overnight fast and resting contents are collected for analysis.
(ii) 100 ml of 7 per cent ethyl alcohol is administered. Samples of gastric contents are collected at an interval of 15 minutes and all the samples are analysed for free and total acidity, peptic activity, presence of bile, blood and mucus.
(iii) The advantages of alcohol test meal are the followings:
(a) More easily administered and prepared.
(b) Consumed better.
(c) The gastric response is more rapid and more intense.
(d) Quick emptying of the stomach.
(e) Specimens are clear and easily analysed.
(iv) The disadvantages of this test are:
(a) Stimulus with alcohol is more vigorous.
(b) Stimulus is not so strictly physiological.
(c) Free acidity levels are higher and normal limits are wider.
Caffeine Stimulation:
(i) The Ryle's tube is introduced into the stomach after overnight fast and the resting gastric contents are collected and analysed.

(ii) Caffeine sodium benzoate (500 mg dissolved in 200 ml of water) is administered orally. Samples of stomach contents are collected at an interval of 15 minutes and analysed for free and total acidity, peptic activity, presence of bile, blood and mucus.
(iii) Advantages of this stimulation is similar to that of alcohol stimulation.

C. Histamine Stimulation test:

Histamine is a powerful stimulant for the secretion of HCl in the normal stomach. It increases the cAMP level which causes the increased secretion of highly acidic gastric juice with low pepsin content.

(i) Standard histamine test:

(a) The Ryle's tube is passed into the stomach after overnight fast and the stomach contents are collected for analysis.
(b) A subcutaneous injection of histamine (0.01 mg/kg body weight) is inserted. 10 ml stomach contents are collected at an interval of 10 minutes for one hour and samples are analysed for free and total acidity, peptic activity, presence of bile, blood and mucus.
(c) Achylia gastrica ("true" achlorhydria) is indicated by the absence of free HCl in the secretion after histamine administration. More juice may be secreted in duodenal ulcer.

(ii) Augmented histamine test:

It is a more powerful stimulus test and it shows an inability to secrete acid. Larger doses of histamine sometimes causes an unwanted severe reactions.
(a) The Ryle's tube is introduced after an overnight fast and the gastric contents are collected for analysis. The resting contents are collected at an interval of 20 minutes for an hour. Halfway of this period, 4 ml anthisan is given intramuscularly.
(b) At the end of the hour, histamine (0.04 mg histamine acid phosphate per kg body weight) is given subcutaneously and gastric contents are collected at an interval of 15 minutes for one hour for analysis.
(c) In pernicious anemia, no free HCl is secreted after histamine stimulation. In duodenal ulcers, higher values of acid are obtained.
(d) Recently, histalog is used in place of histamine. No side effects like histamine are observed by its use. The recommended dose of histamine is 10 to 50 mg. This histalog is highly effective in stimulating gastric secretion.

D. Insulin Stimulation Test:

Hypoglycemia due to insulin administration is an active stimulus of gastric acid secretion. The blood sugar level below 45 mg per cent is essential for a reliable test.
(i) The Ryle's tube is passed into the stomach after an overnight fast and the stomach is made empty.
(ii) 15 units of soluble insulin is injected intravenously and about 10 ml gastric contents are collected at an interval of 15 minutes for 2 ½ hours. The samples are analysed for free and total acidity, peptic activity, and presence of blood, bile, starch. Starch should not be present.
(iii) In duodenal ulcer, acid level is more in response to insulin. The concentration of free acid may be over 100 mEq/litre. After vagotomy no response of insulin is found and the gastric acidity remains at 15 to 20 mEq/litre before and after insulin injection.

E. Pentagastrin Test:

Pentagastrin is a synthetic peptide and it is butyl-oxy-carbonyl β-alanine. It is an active stimulator.

(i) The Ryle's tube is passed into the stomach after an overnight fast and the resting contents are completely removed. After emptying the stomach two 15 minute specimens are collected to have the "basal secretion".

(ii) Pentagastrin (6μg/kg body weight) is injected subcutaneously and specimens are collected at an interval of 15 minutes for analysis.

(iii) The normal basal secretion rate is 1 to 2.5 mEq/hour. The maximum secretion in normal person after pentagastrin stimulus varies from 20 to 40 mEq/hour.

(iv) In duodenal ulcer, the range is 15 to 83 mEq/hour. This test is of little value in gastric ulcer. The "true" achlorhydria is found in cancer of the stomach. The reduced acid level is observed in acute gastritis. The "true" achlorhydria is also noted in Pernicious anemia. The Zollinger- Ellison syndrome is characterised by a high basal secretion usually above 10 mEq/hour and no further rise is found after giving Pentagastrin.

This syndrome is characterised by peptic ulcer, gastric hyper-secretion and diarrhoea in patient with "gastrin". This syndrome is also accompanied by parathyroid adenomas with hyperparathyroidism. The secretion of pepsin occurs after stimulation with pentagastrin.

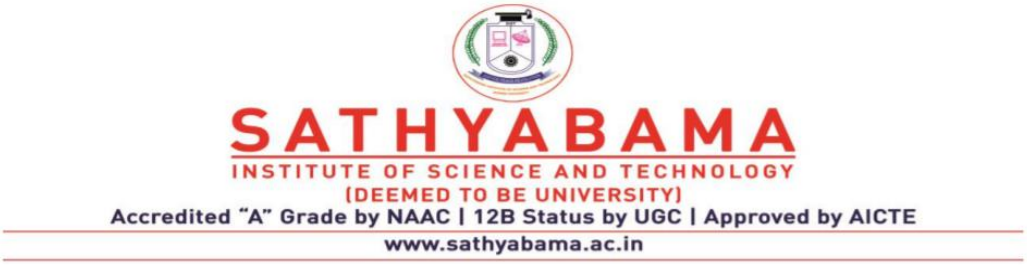
Test # 4. Tubeless Gastric Analysis:

The modified test is done with the introduction of "DiagnexBlue" prepared by reacting carbacrylic cation exchange resin with "Azure A" an indicator. The hydrogen ions of the resin is exchanged with "Azur A" ions.

The reaction is reversed in the stomach when acid is in a concentration having pH less than 3.0. The indicator "Azur A" is released by the action of acid. The released one is absorbed in the small intestine and excreted in the urine, the colour of which is matched with known standards.

This test is valuable if it is used as "screening test" only. A positive result indicates the secretion of acid by the stomach. A negative result is an unreliable indicator of "true" achlorhydria.

This test is not reliable in patients suffering from renal diseases, urinary retention, malabsorption, pyloric obstruction. Vitamin preparation should not be taken on the day proceeding the test which may contain substances decolorized by vitamin C.



SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLGOY

UNIT – V - CLINICAL ENZYMOLOGY SBC3104

UNIT 5 CLINICAL ENZYMOLOGY

Functional and non- Functional plasma enzymes.Isoenzymes with examples. Enzyme patterns in acute pancreatitis, liver damage, bone disorder, myocardial infarction and muscle wasting.

UNIT V

UNIT 5 CLINICAL ENZYMOLOGY

Functional and non- Functional plasma enzymes.Isoenzymes with examples. Enzyme patterns in acute pancreatitis, liver damage, bone disorder, myocardial infarction and muscle wasting.

CLINICAL ENZYMOLOGY

Plasma Enzymes

Blood plasma contains many enzymes, which are classified into functional and non-functional plasma enzymes.

Differences between functional and non-functional plasma enzymes :-

Functional plasma enzymes

Concentration in plasma Present in plasma in higherconcentrations in comparison to tissues

Function Have known functions

The substrates Their substrates are always present in the blood

Site of synthesis Liver

Effect of diseases Decrease in liver diseases

Examples Clotting factors e.g. prothrombin, Lipoprotein lipase and pseudocholine esterase

Non-functional plasma enzymes

Concentration in plasma : Normally, present in plasma in very low concentrations in comparison to tissues

Function No known functions

The substrates Their substrates are absent from the blood

Site of synthesis Different organs e.g. liver, heart, brain and skeletal muscles

Effect of diseases Different enzymes increase in different organ diseases

Examples ALT, AST, CK, LDH, alkaline

phosphatase, acid phosphatase

and amylase,

Sources of non-functional plasma enzymes :

1. Increase in the rate of enzyme synthesis) e.g. bilirubin increases the rate of synthesis of alkaline phosphatase in obstructive liver diseases.

2. Obstruction of normal pathway e.g. obstruction of bile ducts increases alkaline phosphatase.

3. Increased permeability of cell membrane as in tissue hypoxia.

4. Cell damage with the release of its content of enzymes into the blood e.g. myocardial infarction and viral hepatitis.

Medical importance of non-functional plasma enzymes :

Measurement of non-functional plasma enzymes is important for:

1. Diagnosis of diseases as diseases of different organs cause elevation of different plasma

enzymes.

2. Prognosis of the disease; we can follow up the effect of treatment by measuring plasma

enzymes before and after treatment.

Examples of medically important non-functional plasma enzymes :

1. Amylase and lipase enzymes increase in diseases of the pancreas as acute pancreatitis.

2. Creatine kinase (CK) enzyme increases in heart, brain and skeletal muscle diseases.

3. Lactate dehydrogenase (LDH) enzyme increases in heart, liver and blood diseases.

4. Alanine transaminase (ALT) enzyme, it is also called serum glutamic pyruvic transaminase (SGPT). It increases in liver and heart diseases.

5. Aspartate transaminase (AST) enzyme, it is also called serum glutamic oxalacetic transaminase (SGOT).

It increases in liver and heart diseases.

6. Acid phosphatase enzyme increases in cancer prostate.

7. Alkaline phosphatase enzyme increases in obstructive liver diseases, bone diseases and

hyperparathyroidism.

Classification of Enzymes

Enzymes are classified according to the type of reaction they catalyze into six groups:

1. **Oxido-reductases**

These are enzymes that catalyze oxidation-reduction reactions.

Oxido-reductases are further classified into five subgroups:

A- Oxidases

These are enzymes that catalyze direct transfer of hydrogen to oxygen and form water e.g. cytochrome oxidase and ascorbic acid oxidase.

B- Aerobic Dehydrogenases

These are enzymes that catalyze direct transfer of hydrogen to oxygen and form hydrogen peroxide (H2O2) e.g. L-amino oxidase and D-amino acid oxidase.

C- Anaerobic dehydrogenases

These are enzymes cannot transfer hydrogen directly to oxygen but hydrogen is indirectly transferred to oxygen through many hydrogen carriers e.g. glucose-6-phosphate dehydrogenase and succinate dehydrogenase.

D- Hydroperoxidase

These enzymes use hydrogen peroxide (H2O2) as substrate changing it into water (H2O) e.g.

peroxidases and catalases

E- Oxygenases

These enzymes catalyze direct incorporation of oxygen into substrate. e.g.

i- Dioxygenases (True oxygenases): These enzymes catalyze incorporation (introduction) of two oxygen atoms into substrate e.g. tryptophan pyrrolase enzyme.

ii- Monooxygenases (pseudo-oxygenases or hydroxylases): These enzymes incorporate one oxygen atom into substrate e.g. phenylalanine hydroxylase.

2. **Transferases**

These are enzymes that catalyze transfer of a chemical group from one compound to another. They include:

A- Transaminases

These are enzymes that catalyze transfer of amino group (-NH2) from amino acid to ?-keto acid

producing new amino acid and new keto acid e.g.

i- Alanine transaminase (ALT): It is also called serum glutamic pyruvic transaminase (SGPT).

ii- Aspartate transaminase (AST): It is also called serum glutamic oxalacetic transaminase (SGOT).

B- Acyl transferases

These enzymes catalyze the transfer of acyl (fatty acid) group to compounds. They need Coenzyme-A that acts as a carrier for acyl group e.g. choline acetylase.

C- Methyl transferases

These enzymes transfer methyl group (-CH3) from methyl donor usually active methionine (S-adenosyl methionine) to the substrate e.g. formation of epinephrine (adrenaline) from norepinephrine (noradrenaline).

D- Phosphotransferases

These enzymes catalyze transfer of phosphate group e.g. hexokinase and glucokinase both catalyze transfer of phosphate group from ATP to glucose.

3. **Hydrolases**

These enzymes catalyze cleavage of their substrates by addition of water.

All the digestive enzymes are hydrolases. Hydrolases include:

A- Enzymes hydrolyzing glycosidic link in carbohydrates

These enzymes catalyze hydrolysis of carbohydrates e.g. maltase, lactase, sucrase and amylase.

B- Lipase: Enzyme that hydrolyzes triglyceride into glycerol and three fatty acids.

C- Proteases: Enzymes that catalyze hydrolysis of proteins (proteolytic enzymes). e.g. pepsin and trypsinD- Phosphatases:

These enzymes catalyze hydrolysis of phosphoric acid esters e.g..

i- Phosphomonoesterases that catalyze hydrolysis of phosphoric acid monoesters as glucose-6-phosphatase

ii- Phosphodiesterases that catalyze hydrolysis of phosphoric acid diesters e.g. the enzyme that catalyzes the hydrolysis of cAMP (cyclic adenosine monophosphate) to AMP.

4. **Lyases (desmolases)**

These enzymes catalyze cleavage of substrates or removal of chemical groups by mechanisms other than addition of water i.e. by mechanisms other than hydrolysis.

They include:

A. Aldolase: It is an enzyme that splits aldehyde from alcohol e.g. fructose-1-6-diphosphate aldolase.

B. Dehydratases: These enzymes catalyze removal of water from their substrates e.g. fumarase and carbonic anhydrase

C. Decarboxylases:

These enzymes catalyze the removal of CO2 from their substrates.

They need pyridoxal phosphate (PLP) as coenzyme e.g. amino acids decarboxylases as histidine

decarboxylase, which removes CO2 from histidine changing it to histamine

D. Phosphorylases: These enzymes catalyze cleavage of their substrates by addition of phosphoric acid e.g. glycogen phosphorylase.

5. **Isomerases:**

These enzymes catalyze intramolecular rearrangement, so they catalyze conversions between optical, positional and geometric isomers

They include:

A. Aldose-ketose isomerases: These enzymes catalyze interconversion between aldoses and ketoses e.g.

B. Epimerases: These enzymes catalyze interconversion between epimers e.g.

C. Mutases: These enzymes catalyze transfer of chemical group from one position to another in the same compound e.g.

D. Racemases: These enzymes catalyze interconversion between D & L enantiomers e.g.

E. Cis-Trans isomerases: These enzymes catalyze interconversion between cis and trans geometric isomers

6. **Ligases:**

These enzymes catalyze the binding of two molecules to form one molecule.

They need energy which is derived from ATP e.g. glutamine synthetase

Acute pancreatitis

Acute pancreatitis typically presents with severe, constant upper abdominal pain which may radiate through to the back and be associated with nausea and vomiting. As atypical presentations are frequent and there is a wide differential diagnosis, confirmatory tests are required to confirm the diagnosis of acute pancreatitis.[4] Three enzymes derived from pancreatic

acinar cells—amylase, lipase, and the proenzyme trypsinogen—have been tested as biochemical markers of acute pancreatitis; serum amylase is the most commonly used of these in clinical practice.

Amylase

A raised level of serum amylase activity, at least three times the upper limit of normal, supports the diagnosis of acute pancreatitis. Its activity rises quickly within the first 12 hours after the onset of symptoms and returns to normal within three to five days.[5] Serum amylase activities may be normal in 19–32% of cases at the time of hospital admission, as a result of delayed presentation or exocrine pancreatic insufficiency—for example, secondary to chronic alcohol abuse.[6,7] Hypertriglyceridaemia competitively interferes with the amylase assay and can produce falsely low results,[8] although this is variable and can be modulated by the use of lipid clearing agents. Conversely, serum amylase activities can be increased in other intra-abdominal inflammatory conditions and salivary gland pathologies, and also where there is decreased renal clearance because of renal impairment or macroamylasaemia (where amylase is bound to immunoglobulins or polysaccharides to form large molecular weight complexes[8]).

The sensitivity and specificity of amylase as a diagnostic test for acute pancreatitis depends on the chosen threshold value. By raising the cut off level to 1000 IU/l (more than three times the upper limit of normal), amylase has a specificity approaching 95%, but a sensitivity as low as 61% in some studies.[8]

Lipase

Compared with serum amylase, serum lipase activity remains increased for longer (up to 8 to 14 days), thereby giving greater sensitivity in patients with a delayed presentation. Pancreatic lipase activities are more than four times that of amylase and as such are less likely to be affected by chronic pancreatic insufficiency.[9] The recent UK guidelines for the management of pancreatitis state: “Where lipase is available it is preferred for the diagnosis of acute pancreatitis.”[10] Lipase is not specific to the pancreas, and serum activities may also be raised in other intra-abdominal pathologies or in renal insufficiency. Hypertriglyceridaemia does not interfere with laboratory measurement, but drugs such as frusemide can increase serum activity. The diagnostic accuracy of lipase appears to be better than that of amylase. At a cut off activity of 600 IU/l, most studies have reported specificities above 95%, with sensitivities ranging between 55% and 100%.[8,11]

LIVER DISEASE:

Aminotransferases/Transaminases

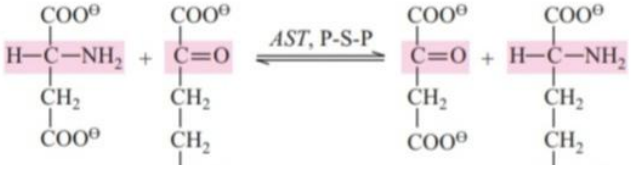
The transaminases are enzymes involved in the transfer of an amino group from a 2-amino- to a 2-oxoacid: they need the cofactor, pyridoxal phosphate for optimal activity. They are widely distributed in the body.

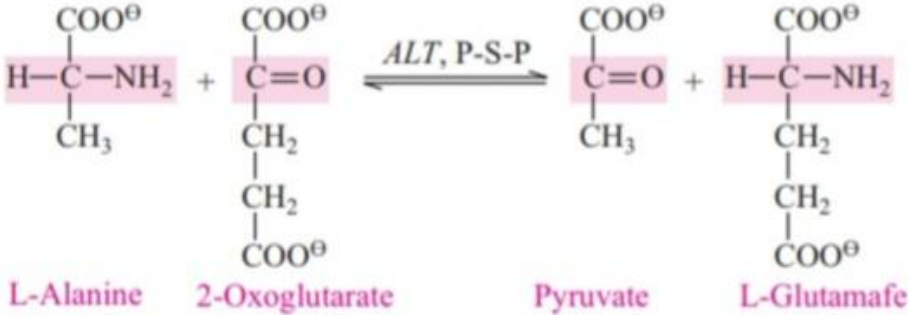
The 2-oxoglutarate/L-glutamate couple serves as one amino group acceptor and donor pair in all amino-transfer reactions; the specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group.

ALT catalyzes the analogous reaction:

The reactions are reversible, but the equilibrium of AST and ALT reactions favor formation of aspartate and alanine respectively.

• In the liver, the concentration of ALT per unit weight of the tissue is more than AST.





• AST and ALT enzymes are more important in assessing and monitoring the degree of liver cell inflammation and necrosis.

• Elevated plasma ALT are considered to be relatively specific for liver disease.

• AST may be elevated in other forms of tissue damage, such as myocardial infarction, muscle necrosis and renal disorders.

• In liver disease, the ALT level is increased markedly compared to AST.in acute viral hepatitis there is a 100-1000 times increase in both ALT and AST but ALT level is increased more than that of AST.

(a) Aspartate Transaminase

Clinical Significance

Normal values of AST: Male: <35 U/L = <0.60 mkat/ Female: <31 U/L = <0.53 mkat/L

(b) Alanine Transaminase

Clinical Significance

Normal values of ALT: Male: <45 U/L = <0.77 mkat/L

Female: <34 U/L = <0.58 mkat/L

BONE DISORDER:

1. **Alkaline phosphatase**
   Rises in Tickets, osteomalaria, hyperparathyroidism and in paget's disease. Also rises in primary and secondary malignancies of bones.
2. **Acid phosphatase**

   Highly increased in bony metastasis of carcinoma prostate.

MYOCARDIAL INFARCTION:

• Commonly known as a heart attack.

• occurs when blood flow stop to a part of the heart causing damage to the heart muscle.

• The most common symptom is chest pain or discomfort which may travel into the shoulder, arm, back, neck or jaw.

• Often it is in the center or left side of the chest and last for more than a few minute.

Diagnosis of acute myocardial infarction (AMI)

• The diagnosis of AMI is usually predicted on the WHO criteria of chest pain, ECG changes and increase in biochemical markers of myocardial injury.

• Half of the patients with " typical " sympy do not have AMI.

• In contrast biochemical marken have excellent sensitivity diagnosing AMI.

Serum enzymes in Acute myocardial infarction

Enzyme assays routinely carried out for the diagnosis of A cute myocardial infarction are :

• Creatine phosphokinase

• Aspartate Transaminase

• Lactate dehydrogenase

• Troponins

• Myoglobin.

1.Creatine kinase (CK / CPK)

• Creatine + ATP < --------- > phosphocreatine + ADP

(phosphocreatine – serve as energy reserve during muscle contraction)

• It is a enzyme found primarly in the heart and skeletal muscle and to a lesser extent in the brain but not found at all in liver and kidney.

• Catalyses the transfer of phosphate between creatine and ATP / ADP.

• Provides rapid regeneration of ATP when ATO in low.

Creatine kinase (Ck/ CPK)

Normal range for total CK

Male : 46-171 U/ L = 0.78-2.90ukat/ L

Female : 34-145 U/L = 0.58-2.47 ukat/ L

Creatine kinase isoenzymes

There are three isoenzymes

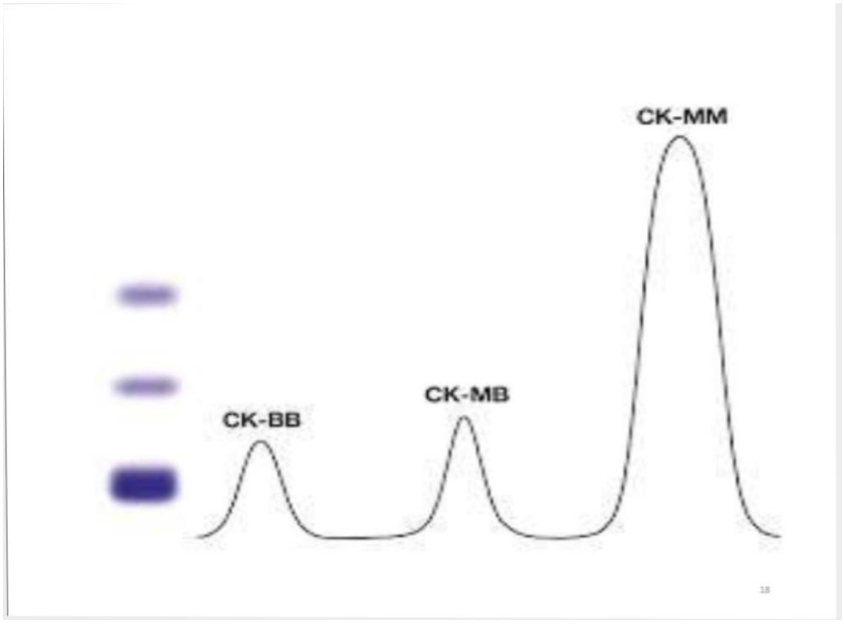
• measuring then is of value in the presence of elevated levels of CK on CPK to determine the source of the elevation.

• Each isoenzymes is a dimer composed of two promoters M(for muscle) and B (for brain) .

• These isoenzymes can be separated bye electrophoresis by ion exchange chromatography.

Three main CK isoenzymes with two polypeptide chain B or M

Type	Composition	Comment
Skeletal Muscle	98% CK-MM 2% CK-MB	Elevated in muscle disease
Cardiac muscle	70-80% CK-MM 20-30% CK-MB	Cardiac muscle has highest amount of CK-MB
Brain	CK-BB	
Plasma	Mainly CK-MM	



Creatine kinase diagnosis

After myocardial infractions serum value is found to increase within 3-6 hours, reaches a peak level in 2-4 days.Normal value serum activity varies from 10-501U/L at 30°C.CK is a sensitive indicator in the early stages of myocardial ischemia. No increase in activity is found in heart failure and coronary insufficiency. In acute MI, CPK usually rise faster than SGOT and return to

normal faster than the SGOT.

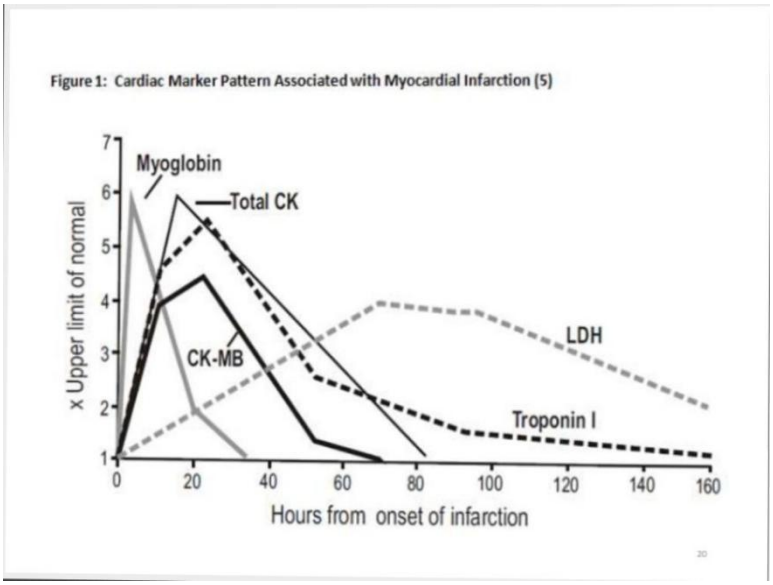
2. Aspartate Ami no Transferase(AST)

• It is also called as Serum glutamate oxalo acetate Transaminase

• The level is significant elevated in acute MI

• Normal value : 0-41IU/L at 37°C

• In acute MI serum activity rises sharply within the first 12 hours, with a peak level at 24 hours or over and returns to normal within 3-5 days.



• The rise depends on the extent of infarction.

3 . Lactate dehydrogenase

In acute MI

• the serum activity rises within 12 to 24 hours, attains a peak at 48 hours reaching about 1000IU / L and then return gradually to normal from 8th to 14th day.

• The magnitude of rise is proportional to the extent of myocardial infractions.

• Serum LDH elevation may persist for more than a week after CPK and SGOT levels have returned to normal levels.

Isoenzymes of LDH

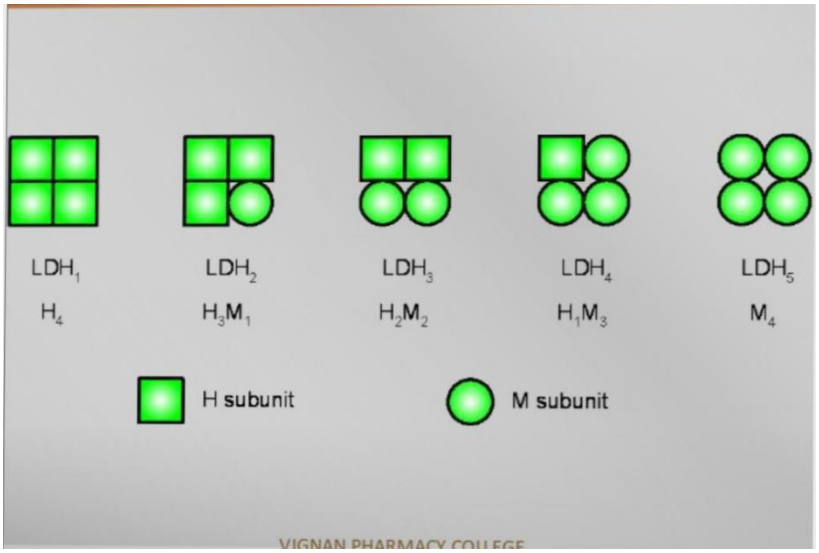
• LDH enzymes is tetramer with 4 subunits.

• The subunit may be either H (heart) or M (muscle) polypeptide chain.

• These two chins are the product of 2 different genes.

• Although both of them have the same molecular weight, there are minor amino acid variations.

• There can be 5 possible combination: H4, H3M1, H2M2, H1M3, M4, these are 5 different types of isoenzymes seen in all individuale.



MUSCLE ENZYMES

Creatine Kinase CK is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle.The concentration gradients between some human tissues and serum for creatine kinase. The concentration gradient is logarithmic

Clinical significance

Normal range for total CK:

Male : 46-171 U/L= 0.78-2.90 µkat/L

Female: 34-145 U/L= 0.58-2.47 µkat/L

Serum CK activity is greatly elevated in all types of muscular dystrophy. In Progressive muscular dystrophy (particularly Duchenne sex-linked muscular Dystrophy), enzyme activity in serum is highest in infancy and childhood (7-10 Years of age) and may increase long before the disease is clinically apparent. serum CK activity characteristically falls as patients get older and as the mass functioning muscle diminishes with the progression of the disease. About 50%-80% of the asymptomatic female carriers of Duchenne dystrophy show threefold to six-fold increase of CK activity. Quite high values of Ck are noted in viral myositis, polymyositis and similar muscle disease. However in neurogenic muscle disease, such as:

(a) Myasthenia gravis
(b) Multiple sclerosis© Polimyeltis

(c) Parkinsonism

Serum enzyme activity is normal

Lactate Dehydrogenase

Catalyses the reversible interconversion of lactate and Pyruvate. The enzyme is widely distributed in the body, with high concentrations In cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes:Measurement of plasma total LD activity is therefore a non-specific marker of Cell damage**.**

LD has a molecular weight of 134 kDa and is composed of four peptide chains Of two types:

M (or A)

H (or B)

Each under separate genetic control

The subunit compositions of the five isoenzymes are listed below in order of Their decreasing anodal mobility in an alkaline medium.

LD-1 (HHHH; H4) = migrates fastest towards the anode

LD-2 (HHHM; H3M)

LD-3 (HHMM; H2M2)

LD-4 (HMMM; HM3)

LD-5 (MMMM; M4)

Clinical significance

Normal range of total LDH: 180-360 U/L= 3.1-6.1 μkat/L

It is increased in plasma in Myocardial injury, acute leukaemia, generalized Carcinomatosis and in acute hepatitis. Estimation of its isoenzymes in more Useful in clinching diagnosis between hepatic disease and Myocardial Injury.