LIVER FUNCTION TESTS PROTOCOL AND SIGNIFICANCE.

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Abstract
Biochemistry is the fascinating science, the Basis of Medicine, extensively useful to understand the diseases at molecular level.
This paper aims in giving state-of-the-knowledge review on liver functions and tests by descriptive diagrams and flow charts.
The article gives a bird’s eye view on various basic Biochemical aspects on functions of liver and metabolic reactions involved along with panel of tests.
Imbalance /dysfunction / malfunctions of metabolic reactions of liver lead to diseases with clinical manifestations.
Disorders, Biochemical basis of the disorders, salient biochemical parameters, their levels in health and disease at one roof, aids the budding doctors to diagnose for onward appropriate treatments in treating the diseases.

Introduction:-
Liver Function Tests
Liver function tests (LFT) are simple blood tests an effective modality, helps to screen and to detect hepatic dysfunction and liver disorders. Diagnosis of liver disorders involves a range of tests which are grouped under liver function tests.

The liver (hepar) is a functionally complex versatile vital body organ which plays a major role in detoxification and metabolism. Medication and other substances including toxic substances enter liver through the portal vein for utilization and detoxification.
Humans cannot survive without it as it is involved in important physiological functions, synthesizes many compounds and enzymes that pass into the bloodstream which are essential for various metabolic activities.

Functions Of Liver
Liver being functionally complex organ of the body plays vital biochemical role in variety of functions like detoxification, storage, secretion, digestion, synthesis, excretion, erythropoesis, homeostasis and metabolic functions.

Functions of the liver are depicted in Fig. 1.1.
Hyperbilirubinemia
Bilirubin, end product of hemoglobin catabolism is responsible for the yellow color of bile.
1. Normal level of total Bilirubin is up to 1.0 mg/dl.
2. An excess amount of bilirubin in the body above 1 mg/dl is known as Hyperbilirubinemia.
3. Latent Jaundice is mild elevation of bilirubin (1-2 mgs/dl) where yellow discoloration is not marked.
4. When the bilirubin in the body exceeds more than 2-3 mg/dl, it diffuses to sclera, conjunctiva and mucous membrane giving a yellow coloration JAUNDICE/ICTERUS.
5. Sclera has a particular affinity for bilirubin due to their high elastin content. The presence of scleral icterus indicates a serum bilirubin of at least 3 mg/dl.

Jaundice is not a disease; it is a disorder of liver /Gall bladder / hemolytic disorder or together with symptoms or clinical signs due to many other conditions.
Jaundice can be caused by either of the following reasons:
1. The overproduction of bilirubin by excessive breakdown of RBC (Haemolytic Jaundice)
2. Failure of the liver cells to metabolize or excrete the bilirubin produced (Hepatic Jaundice)
3. Blockage of the bile ducts affecting the flow of bile into intestine (Obstructive Jaundice)
4. Poisoning
5. Viral hepatitis (hepatitis B)
6. Excess alcohol ingestion
7. Autoimmune disorders

**Haemolytic Jaundice (Pre Hepatic Jaundice)**

Haemolytic jaundice (See Fig. 2.3) is due to overproduction of bilirubin caused by the destruction of unusually large number of red blood cells. This may occur in hemolytic diseases such as Malaria, Thalassemia, Leukemia, Hodgkin’s disease, Erythroblastosis fetalis of newborn. In these condition the liver cannot conjugate and excrete the bilirubin which is formed more rapidly in large amounts. Haemolytic jaundice is with excessive amounts of unconjugated bilirubin in the plasma and without bile pigments in the urine (Bilirubinuria negative).

Van den Bergh test shows indirect positive reaction showing increase in unconjugated (Indirect) bilirubin with normal ALT and AST enzyme levels.

**Findings**

1. Increased levels of serum unconjugated bilirubin
2. Increased levels of Urobilinogen in urine
3. Increased levels of stercobilinogen in feces (dark colored stools)
4. Normal ALT and AST enzyme levels.

**The main causes are:**

1. Hemoglobin abnormalities
2. Hemolytic diseases
3. Drugs and chemical substances which cause damage to RBC
4. G6PD deficiency
5. Auto immune disorders
6. Genetic disorders
7. Erythroblastosis fetalis of newborn
Hepatic Jaundice (Infective Jaundice)
Hepatic jaundice (See Fig. 2.4) occurs due to the defect in liver that prevents bilirubin conjugation. Levels of bilirubin increase when the liver has lost at least half of its excretory capacity. It may be either due to impaired uptake of bilirubin by hepatocytes / failure of conjugation / impaired secretion. Hepatic jaundice occurs when the primary problem causing the jaundice resides in the liver (intrinsic liver defect or disease) that result in elevations of unconjugated bilirubin. In healthy people, conjugated bilirubin is virtually absent from serum because of the rapid process of bile secretion.

Van den Bergh test shows bi-phasic reaction with increase in both conjugated (Direct) and unconjugated (Indirect) bilirubin. There is increase in ALT and AST levels with moderate increase in ALP.
Findings
Increase in both conjugated (Direct) and unconjugated (Indirect) bilirubin in serum.
Excretion of bilirubin and urobilinogen in urine.
Increase in ALT and AST levels with moderate increase in ALP due to release from the damaged liver cells.

The main causes are--
1. Hepatitis virus- HAV, HBV, HCV etc.
2. Cholestatic diseases.
3. Toxic Hepatitis-Use of drugs like tetracycline, valproate, salicylates, etc.
4. Amyloidosis.
5. Cirrhosis.
6. Alcoholism.
7. Drugs
8. Hepatocellular carcinoma and metastasis in liver.

Obstructive Jaundice (Post Hepatic Jaundice)
Obstructive jaundice (See Fig. 2.5.) is due to blockage of the bile ducts that decreases the flow of bile and bilirubin from the liver into the intestines. Physical obstruction to the flow of bile can be due to cancers, gallstones or inflammation of the bile ducts that prevent the flow of conjugated bilirubin. Biliary obstruction causes conjugated Hyperbilirubinemia. Van den Bergh test shows Direct positive showing increase in conjugated (Direct) bilirubin. Increased levels of ALP and γGT as compared to increase in AST and ALT. Obstruction decrease the absorption of...
vitamin K and therefore increase Prothrombin time. Stool becomes clay-colored as stools loses source of normal pigmentation from bile pigments due to obstruction

**Findings**
1. Increase in serum conjugated (Direct) bilirubin
2. Excretion of excessive amounts of conjugated bilirubin
3. Clay colored stools due to absence of stercobilinogen in feces
4. Absence of bile in the intestine leads to steatorrhea
5. Increased levels of ALP and γGT as compared to increase in AST and ALT levels.

**The main causes are--**
1. Gallstones.
2. Cancer of biliary system.
4. Intra hepatic viral cholestasis.

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**Fig 1.4:** Obstructive jaundice.
Disorders of liver:-
The liver becomes unable to function normally in many conditions as mentioned below.
1. **Hepatitis**: Inflammation of the liver is usually caused by viruses like hepatitis A, B, C, D and E. Heavy drinking, drugs, allergic reactions can also cause Hepatitis.
2. **Cirrhosis**: Cirrhosis is a clinical condition in which "scar tissue replaces normal healthy liver tissue." As a result, it blocks the flow of blood through the organ and prevents its normal functioning.
3. **Liver cancer**: The most common type of liver cancer, hepatocellular carcinoma, almost always occurs after cirrhosis.
4. **Liver failure**: Liver failure has many causes including infection, genetic diseases and excessive alcohol consumption.
5. **Ascitis**: As cirrhosis results, the liver leaks fluid (ascites) into the belly, which becomes distended and heavy. Infection with viral hepatitis A, B, C, D and E also causes ascitis.
6. **Obstruction of bile duct**: If a gallstone becomes stuck in the bile duct objecting the flow of bile from the liver, hepatitis and bile duct infection (cholangitis) can cause inflammation and scarring of the bile ducts in the liver.
7. **Hemochromatosis**: Hemochromatosis allows iron to deposit in the liver, damaging the hepatocytes affecting its normal functions.
8. **Fatty liver**: Fatty liver is accumulation of fat more than 5% of the organ's weight in the liver which results in inflammation and damage to the liver.
9. **Wilson disorder**: Accumulation of copper in liver leads to hepatolenticular degeneration.
10. **Drugs induced liver disorders**: Continuous use of anti-seizure medications, antibiotics, statins, cardiovascular drugs, antidepressant drugs and history of blood transfusions results in liver disorders.
11. **Glycogen storage disorders**: Glycogen storage disorders cause varying degrees of liver enzyme abnormalities.

Liver Function Tests And Significance

**Liver Function Tests (LFT)**
Since liver performs a variety of functions, no single test is sufficient to provide complete estimate of function of liver. Choice of assay of biochemical parameters under LFT is very important for differential diagnosis. Several biochemical tests are useful in evaluation, management and treatment modality and to monitor progression of hepatic diseases.
Liver function tests are broadly classified into group tests

**GROUP I TESTS**
- BLOOD--BILIRUBIN
- URINE--BILESALTS, BILEPIGMENTS, UROBILINOGEN

**GROUP II TESTS**
- TOTAL PROTEIN, ALBUMIN, GLOBULIN, A:G RATIO, PROTHROMBIN TIME, CERULOPLASMIN, α1

**GROUP III TESTS**
- ALT, AST, ALP, γGT, LDH

**GROUP IV TESTS**
- IMMUNOLOGY BLOOD TESTS, VIRUSES ANTI BODIES
- AUTO ANTIBODIES
- CT, MRI, USG. CHOLECYSTOGRAPHY, LIVER BIOPSY

Group IV tests are special tests
Significance of Liver function tests

**Fig. 2.7:** Significance of Liver Function Tests.

**Routinely done tests under LFT are:**

**Group 1a-- Excretory functions of liver**

**Serum Bilirubin**

Formation of bilirubin and its excretion

1. Bilirubin is the principal degradation product of heme.
2. The hemoglobin after approximately 120 days is phagocytosed by macrophages, and split into heme, Iron and globin portions. Heme gets degraded to Biliverdin which is subsequently converted to Bilirubin by the enzyme bilirubin reductase.
3. This bilirubin then binds albumin (carrier protein) and is taken up by the liver.
4. In liver, bilirubin undergoes conjugation with glucuronic acid to form bilirubin monoglucuronide and bilirubin diglucuronide (conjugated bilirubin-Direct bilirubin) by the enzyme glucuronyl transferase.
5. Conjugated bilirubin being water soluble enters intestine along with bile for its further metabolism in intestine.
6. They are converted by intestinal flora to Urobilinogen (UBG) and stercobilinogen (SBG), colorless compounds which are later oxidized to urobilin and stercobilin which imparts the normal color to urine and faeces respectively.
7. About 20% of UBG is reabsorbed into liver by portal blood and is excreted in urine.
8. Bilirubin, if not conjugated will be present as unconjugated bilirubin (Indirect bilirubin).
9. Unconjugated bilirubin being water insoluble is not secreted into bile so gets regurgitated into general circulation.

**Fig. 1.6:** Normal metabolism of Heme.
Van den Bergh reaction
Van den Bergh reaction is used in estimation of serum Bilirubin. Van den Bergh reagent contains sulfanilic acid and sodium nitrite (Diazotized sulfanilic acid).

Van den Bergh- Direct positive
When Van den Bergh reagent is added to serum containing an excess of conjugated bilirubin (bilirubin diglucuronid), a reddish violet color results within 30 seconds. This is called the Van den Bergh Direct positive—Obstructive jaundice.

Van den Bergh- Indirect positive
When the reagents are mixed with serum containing an excess of unconjugated bilirubin. No color develops until alcohol is added whereupon the reddish-violet color appears this is called the Van den Bergh Indirect positive--Haemolytic jaundice.
Van den Bergh- Biphasic
When the reagents are mixed with serum reddish violet color produced immediately within 30 seconds and on addition of alcohol the color intensifies this is called the Van den Bergh Biphasic - Infective (Hepatic) jaundice.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolytic jaundice</td>
<td>Indirect positive</td>
</tr>
<tr>
<td>Hepatic jaundice</td>
<td>Biphasic</td>
</tr>
<tr>
<td>Obstructive jaundice</td>
<td>Direct positive</td>
</tr>
</tbody>
</table>

Normal range:

<table>
<thead>
<tr>
<th>Test</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bilirubin</td>
<td>Up to 1.0 mg/dl</td>
</tr>
<tr>
<td>Direct Bilirubin</td>
<td>0.0 to 0.2 mg/dl</td>
</tr>
<tr>
<td>Indirect Bilirubin</td>
<td>0.2 to 0.8 mg/dl</td>
</tr>
</tbody>
</table>

TO NOTE
Serum and plasma samples must be kept in dark for bilirubin estimation
Bilirubin being light sensitive, photo degradation of bilirubin will result in significant lowering of plasma bilirubin level.
Determination of the level of bilirubin in the blood is of value in detecting elevated bilirubin levels at the earliest stages before jaundice appear.

Ratio of Direct Bilirubin and Indirect Bilirubin are used for differential diagnosis of different types of jaundice.

Urine Analysis
Urine is analyzed for bile pigments, bile salts and urobilinogen in jaundice. Presence/Absence of these in urine helps to differentiate different types of jaundice.

Urine Bilirubin
Small amounts of bilirubin may be present in the urine of healthy individuals which are not detected by standard laboratory tests. Therefore, it is generally assumed that no bilirubin is present in the urine of healthy individuals.
The condition in which high levels of bilirubin are detected in the urine is called bilirubinuria.

In obstructive jaundice conjugated bilirubin is regurgitated to circulation due to obstruction in the flow of bile and is excreted in urine
Detection of Urine Bilirubin
Urine Bilirubin is detected by Fouchet’s test.
Fouchet’s reagent contains ferric chloride in Trichloro acetic acid (TCA)
Urine Bile Salts
Bile salts are derived from Cholesterol, excreted through bile, usually not excreted in urine. They cause emulsification of fats thereby aiding in digestion of fats. They are detected in obstructive jaundice due to regurgitation of bile into systemic circulation and hence excreted in urine. They can be detected sometimes in Hepatic jaundice when there is inflammation in liver which obstructs the biliary channels, thereby leading to regurgitation of bile into systemic circulation.

Detection of Urine Bile salts
Urine bile salts are detected by Hay's Sulphur test.

Urine Urobilinogen
Normally only traces of Urobilinogen is present in urine. In the intestine, bilirubin by bacterial action forms colorless bilinogens- Urobilinogen, mesobilinogen and stercobilinogen.

20% of Urobilinogen is reabsorbed from the intestine by enterohepatic circulation. A small fraction of urobilinogen enters the systemic circulation and is excreted in urine. Stercobilinogen is mostly excreted through feces.
A. Detection of urobilinogen:
Urine urobilinogen is detected by Ehrlich test.

- In Haemolytic jaundice, an increase in excretion of urobilinogen is found.
- In Hepatic jaundice, excretion of urobilinogen is either normal or increased depending on the extent of damage to hepatic cells.
- In obstructive jaundice, there will be no urobilinogen excretion.

Fecal Stercobilinogen
1. Normal color of the feces is due to the presence of Stercobilin.
2. Intestinal bacteria deconjugate the conjugated Bilirubin.
3. Free bilirubin is reduced to Urobilinogen.
4. Further reduction of urobilinogen leads to formation of Stercobilinogen which is oxidized to Stercobilin that imparts the normal color to feces.

Group 1b: Test For Metabolic Capacity Of Liver
This test is done to find abnormalities in the conversion of ammonia to urea.

Blood Ammonia
Ammonia, the catabolic end product of amino acids (Proteins) is a neuro toxic substance. It is also formed due to bacterial action in the intestines. Most of this ammonia is then absorbed by the intestines and goes into the portal circulation, where normally the liver converts it to urea and it is excreted by the kidneys. Ammonia being highly toxic is converted to urea in liver (urea cycle).

<table>
<thead>
<tr>
<th>Normal levels of Ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
</tr>
<tr>
<td>Children</td>
</tr>
<tr>
<td>Neonates</td>
</tr>
</tbody>
</table>

Hyperammonemia:
Hyperammonemia is a metabolic condition characterized by elevated levels of ammonia in the blood, which leads to highly fatal ammonia intoxication resulting in hepatic encephalopathy. Hyper ammonemia is seen in portal hypertension, Cor pulmonale and renal failure.
Lactulose (galactose and fructose) is used in the treatment of hyper ammonemia.

Causes
1. Congenital deficiencies / absence of urea cycle enzymes.
2. Hepatic encephalopathy
3. Reye’s syndrome.
4. Toxic encephalopathy.
5. Cor pulmonale.
6. Renal failure.

Six enzymes are involved in urea cycle to convert ammonia (from protein) and carbon dioxide to urea. Deficient / defective enzyme necessary to convert ammonia into urea leads to urea cycle disorders mainly Hyperammonemia

<table>
<thead>
<tr>
<th>DEFECTIVE ENZYME</th>
<th>DISORDERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Carbamoyl phosphate synthetase (CPS I)</td>
<td>Hyperammonemia type I</td>
</tr>
<tr>
<td>2. Ornithine transcarbamylase (OTC)</td>
<td>Hyperammonemia type II</td>
</tr>
<tr>
<td>3. Argininosuccinic acid synthetase</td>
<td>Citrulinaemia</td>
</tr>
<tr>
<td>4. Argininosuccinase acid lyase</td>
<td>Argininosuccinic aciduria</td>
</tr>
<tr>
<td>5. Arginase</td>
<td>Argininemia</td>
</tr>
</tbody>
</table>

Table 1.1: Disorders of Urea cycle.

All defects in urea cycle leads to ammonia intoxication and leads to hepatic coma and death. Increased entry of ammonia to the brain is a primary cause of neurologic disorders.

**TO NOTE**
1. Ammonia levels show the metabolic capacity of the liver.
2. Ammonia intoxication leads to rapid change in cognitive functions.
3. Symptoms of ammonia intoxication are slurring of speech, blurring of vision, delirium, hepatic coma and death.
4. Blood ammonia level is routinely estimated in liver disease and hepatic encephalopathy with portal hypertension.

**Signs and symptoms of early-onset hyper ammonemia in Neonates**
1. Lethargy
2. Irritability
3. Poor feeding
4. Vomiting
5. Hyperventilation, grunting respiration
6. Seizures

Tests done to detect hyperammonemia in Neonates
1. Ammonia levels by Ion selective electrode method
2. Urea cycle enzymes.
3. Arterial blood gas analysis.
4. Serum amino acid levels.
5. DNA mutation analysis.

**Precautions to be taken in estimation of ammonia .**
1. Fasting sample of venous or preferably arterial blood sample is used for ammonia estimation
2. Blood is collected in chilled heparin vacutainer and transported on ice to the laboratory
3. Plasma is separated and analyzed immediately within 15 minutes of collection to prevent erroneous high values
4. Haemolysed sample gives elevated ammonia levels
Group II Tests
Liver is responsible for the synthesis of many proteins grouped as total proteins as some of which are vital for normal function and some of which can be used as markers of liver disease. The main protein synthesized by the liver is Albumin.

Commonly assayed parameters under synthetic functions of the liver are:
1. Serum Total Proteins
2. Albumin
3. Prothrombin time
4. Ceruloplasmin
5. $\alpha_1$ antitrypsin

Total Protein
Three major fractions of plasma proteins are Albumin, Globulins and Fibrinogen. These main fractions can be separated on subjecting plasma proteins to electrophoresis.
1. Albumin is the major constituent (60%) of plasma proteins synthesized exclusively by hepatic parenchymal cells, performs important functions in the body including maintenance of colloidal osmotic pressure.
2. Globulins comprises many proteins with varieties of functions including immunity
3. Fibrinogen is involved in blood coagulation
4. Most of the plasma proteins except immunoglobulins are synthesized in liver.
5. Normal total protein values are altered in some inflammatory conditions of liver when the liver switches in making other acute phase proteins.
6. Total protein values decrease below normal range in cirrhosis of liver and in other liver diseases due to damaged liver cells.
7. Total protein values can also be decreased below normal range in different clinical conditions associated with nephrotic syndrome, malnutrition and renal disorders.
8. Increased total protein levels are found in multiple myeloma, hyper gammaglobulinemias and hypovolemic states.

Total protein is estimated by Biuret method.

Albumin
1. Albumin is synthesized only in liver. It is a sensitive marker of hepatic function.
2. Decrease/failure of Albumin synthesis occurs due to extensive loss of functioning liver tissue and in some inflammatory conditions with compensatory excess synthesis of globulins
3. Albumin level is decreased in Hepato cellular jaundice & Cirrhosis
4. Albumin level is normal in obstructive and Hemolytic Jaundice
5. Low levels of albumin is also seen in other conditions like nutritional problems, malnutrition and in renal diseases (Albuminuria)

Albumin is estimated by Bromo-cresol green (BCG) method.

TO NOTE
1. Liver produces about 12 gms of albumin per day which accounts to 25% of total hepatic protein synthesis.
2. One of the important function of Albumin is maintenance of colloidal osmotic pressure of blood apart from its transport, buffering and nutritional functions. Hypoprotenemia results in decrease of effective osmotic pressure with accumulation of water in tissues causing edema.
3. Edema is seen in cirrhosis of liver, malnutrition, nephrotic syndrome due to decrease in serum albumin levels

Globulin and A/G Ratio
Globulin fraction significantly increases, most commonly during increased activity of the immune system, such as acute infection, chronic inflammatory disease and multiple myeloma

On electrophoresis plasma proteins are separated into Albumin and globulins
Globulin fraction constitute many proteins including an acute phase proteins separated into four distinct bands namely α1, α2, β and γ bands

**α 1 Globulins** -- α1Antitrypsin, lipoproteins, retinol binding protein, thyroxine binding protein, cortisol binding protein

**α 2 Globulins** --- Prothrombin, Haptoglobulins, Ceruloplasmin

**β Globulins**-- Plasminogen, β lipoproteins, Transferrin, Hemopexin

**γ Globulins** are group of Globulins which includes immunoglobins. These are antigen binding proteins (Antibodies)

They play a major role in defense mechanism of body. The immune system of the human body contains two major components humoral immunity and cell mediated immunity.

**Fibrinogen**-- It is synthesized in liver plays an important role in blood coagulation

Fibrinogen band is observed between the β and γ globulins on electrophoresis.

Globulin = Total Protein - Albumin

Globulin fraction significantly increases in acute infection, chronic inflammatory disease, infectious hepatitis and multiple myeloma due to over activity of the immune system

Albumin levels are decreased in hepato cellular jaundice & cirrhosis reversing the A/G ratio

<table>
<thead>
<tr>
<th>Normal Values of Serum Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total proteins</td>
</tr>
<tr>
<td>Albumin</td>
</tr>
<tr>
<td>Globulins</td>
</tr>
<tr>
<td>Fibrinogen</td>
</tr>
<tr>
<td>A/G Ratio</td>
</tr>
</tbody>
</table>

**Prothrombin Time (PT)**

1. Prothrombin is synthesized in liver, Prothrombin time(PT) indicates the functional status of liver
2. Vitamin K is required for the activation of inactive clotting factors II(Prothrombin), VII, IX and X
3. Prothrombin (coagulation factor II) is cleaved to form thrombin by activated Factor X in the clotting process
4. Thrombin in turn converts soluble fibrinogen into insoluble strands of fibrin, which is involved in coagulation
5. PT is a blood test that measures how long it takes to clot blood
6. PT is prolonged in hepatic Jaundice, cirrhosis and liver disease, due to diminished synthesis of clotting factors
7. PT is also prolonged in obstructive Jaundice due to absence / decreased Vitamin K absorption in the intestine
8. In obstructive jaundice bile salts will not be available in intestine for fat digestion (for biliary emulsification) which affects absorption of fat soluble vit K.

PT is normal in Hemolytic Jaundice.

**Normal range:**
10 - 16 seconds.

**TO NOTE**

Prolongation of the PT is not specific only to liver disease

PT is prolonged in other cases –

1. Warfarin use
2. Vitamin K deficiency, biliary obstruction, malabsorption syndromes, use of antibiotics
3. Abnormality or deficiency or presence of an inhibitor to factors VII, X, Prothrombin, V and fibrinogen
4. Disseminated intravascular coagulopathy (DIC)
5. PT remaining prolonged even after parenteral administration of vit k confirms liver disorder

**Ceruloplasmin**

1. Ceruloplasmin is a blue color protein stores and carries 65% to 90% of the copper found in blood.
2. Ceruloplasmin is the copper containing protein mainly synthesized from parenchymal cells of liver, plays a role in transport of copper
3. It has ferro-oxidase activity which oxidizes iron and incorporates it into transferrin
4. It is required for tyrosinase activity
5. Ceruloplasmin level is decreased in Wilson’s disease due to defect in binding of copper with Apo-ceruloplasmin to form ceruloplasmin.
6. As copper is not excreted, accumulation of copper in liver leads to hepatolenticular degeneration which drastically affects the normal functions of liver

**Normal Levels**
25 – 50 mg/dl

**TO NOTE**
1. Ceruloplasmin is an acute phase protein, increased in inflammatory, infective conditions and hemochromatosis
2. Decreased ceruloplasmin level is seen in long-term liver disease, biliary diseases and biliary cirrhosis
3. Decreased ceruloplasmin level is observed in Wilsons disease
4. Increased ceruloplasmin level is observed in people on estrogen and contraceptive treatment.

**Antitrypsin (AAT)**
1. α 1-antitrypsin is produced in the liver. It is known as Protease inhibitor since it combines with all protease enzymes and inhibits their activity
2. Serine proteases are proteolytic enzymes with amino acid serine in their active center
3. α 1-antitrypsin inhibits all serine proteases like plasmin, thrombin, trypsin, chymotrypsin, elastase etc
4. It protects the lungs from the action of neutrophil elastase (elastase enzyme disrupts connective tissue and cause damage to lung tissue leading to emphysema).

**Normal Levels**
100 -- 200 mg / dl.

**TO NOTE**
1. Low serum α 1-antitrypsin (AAT) level is known to be associated with liver disease and cirrhosis
2. Low AAT causes inflammatory changes in the hepatocytes causing deposition of collagen, enhancing the cirrhosis.
3. Deficiency of α 1-antitrypsin is associated with emphysema, chronic Bronchitis, colds and chronic obstructive pulmonary disease

**Group III Tests-- Liver Enzymes**
Liver enzymes are proteins made by the liver. They are increased in the blood due to damaged cells or cell death. Inflamed or injured liver cells leak higher than normal amounts of certain chemicals, including liver enzymes into the bloodstream, resulting in elevated liver enzymes on blood tests.

Commonly measured liver enzymes are-

**Amino Transfereases (AST and ALT)**
1. Aminotransferase are the enzymes involved in the transfer of amino group from a amino acid to a keto acid to form new amino acid and a new keto acid in the presence of coenzyme Pyridoxal-phosphate(vit B6)
2. Aminotransferase(Transaminases) are the enzymes which play a role in amino acid metabolism and in synthesis of non essential amino acids and proteins
3. There are many Aminotransferase of which Alanine amino transferases (ALT/SGPT) and Aspartate amino transferases (AST/SGOT) are of diagnostic importance.
4. AST and ALT enzymes are present in a wide variety of tissues-including heart, skeletal muscle, kidney and brain in addition to liver.
5. The level of the ALT and AST may be used as a general measure to detect the degree of liver inflammation or damage.
6. They are released into serum in proportion to cellular damage and most elevated in acute phase of cellular necrosis

**Alanine Amino Transferase. ALT(SGPT)**
ALT is exclusively an cytoplasmic enzyme present in high concentrations in liver and to a lesser extent in skeletal muscle, kidney and heart
1. ALT is a relatively specific and sensitive indicator of hepatocellular damage. It is released early in liver damage and remain elevated for weeks
2. Raised ALT levels with much lower rise in AST is usually associated with hepatocellular disease such as cirrhosis, hepatitis and non alcoholic steato hepatitis

**Aspartate Amino Transferase, AST (SGOT)**
1. AST is both mitochondrial & cytoplasmic enzyme, widely distributed with high concentration in cardiac tissues and less in skeletal muscle and liver.
2. AST level in serum is not a highly specific indicator of liver injury as its levels are elevated in cardiac disorders and any injured tissues.
3. Raised AST with normal ALT levels is associated with ischemia, CCF and ischemic necrosis
4. Moderate rise in AST is seen in primary hepatomas and alcoholic hepatitis

**Normal Levels**
- ALT -- 5--40 IU/L
- AST-- 5--45 IU/L

**TO NOTE**
1. In most of the liver diseases ALT (SGPT) activity is higher than AST (SGOT) except in alcoholic Hepatitis, hepatic cirrhosis and liver neoplasia.
2. Viral hepatitis shows increase in both serum AST and ALT levels before the onset of jaundice
3. Increased ALT with little or no raise in AST is suggestive of acute hepatocellular disorder
4. Increase in AST with normal ALT is suggestive of AML
5. It is important TO NOTE that ALT and AST levels do not reflect the function of the liver but along with other organ profile tests and clinical manifestations their levels aids in differential diagnosis of diseases

**Alkaline Phosphatase (ALP)**
Alkaline phosphatase (ALP) is an enzyme present in the canalicular and sinusoidal membranes of the liver, is also active in many other tissues, particularly in bone, intestine and placenta
1. Alkaline phosphatase may be elevated due to hepatitis, gallstones, liver cancer or Paget's disease
2. This enzyme plays an important role in the growth and development of bones and teeth.
3. It is an hydrolase enzyme responsible for removing phosphate groups from many types of molecules including nucleotides, proteins in alkaline pH (9 and 10)
4. High levels of ALP is suggestive of liver injury as the liver synthesizes the highest amounts of this enzyme
5. Six isoenzyme forms of ALP have been identified which differ in their carbohydrate moieties. ALP1 is predominantly liver isoenzyme and ALP2 is predominately bone isoenzyme.
6. A test for alkaline phosphatase isoenzyme may be necessary to distinguish between bone and liver alkaline phosphatase.

Affinity electrophoresis using poly acrylamide gel is used in separation and identification of fraction of ALP isoenzymes to differentiate liver or bone disorders

**Normal Levels**
3 - 13. KA U/L

**TO NOTE**
1. Marked elevation of ALP is typical of obstructive jaundice often with elevated γGT
2. Increased levels of ALP with normal γGT is seen in bone disorders
3. Serum alkaline phosphatase activities are generally higher in children due to increased rates of osteoblastic activity associated with calcification process.
4. In infants, ALP levels are approximately three times more to the upper reference limit of adults
5. Increased at the time of pubertal maturation in adolescence when there is increased bone turnover
6. ALP levels are increased in osteomalacia, bone metastasis, Pagets disease
7. ALP is physiologically increased in the third trimester of pregnancy (produced by the placenta).
8. To determine whether ALP is likely to be of hepatic origin, γ-glutamyl transpeptidase (γGT) and 5-prime-nucleotidase measure helps in differential diagnosis.
9. γGT and 5-prime-nucleotidase(5' nucleotidase) values tend to increase along with the ALP levels in patients with liver disease.
10. Raised ALP levels with normal $\gamma$ GT and 5-prime-nucleotidase indicates bone disorder
11. Combination of tests are useful in the interpretation and differential diagnosis of hepatic disease and bone disorders
12. The tissue source of elevated ALP in serum, can be determined by identifying the individual isoenzyme present in excess by electrophoresis

$\gamma$-Glutamyl transpeptidase (\(\gamma\) GT)

1. \(\gamma\) GT enzyme present of serum appears to originate primarily from the hepatobiliary system, although it is present in renal tubules, liver and pancreatic cells
2. \(\gamma\) GT activity is elevated in response to any hepatocellular injury and all forms of liver disease like- Hepatitis, Cirrhosis, Liver metastasis, Carcinoma and Cholestasis
3. Raised \(\gamma\) GT in patients with chronic liver disease is associated with obstructive jaundice
4. \(\gamma\) GT is involved in the transfer of amino acids across the membrane. It transfers the glutamyl moiety to variety of acceptor molecules

\(\gamma\) GT is raised in patients following alcohol ingestion and alcoholic liver disease

Normal Levels
Adult male: 6-45 IU/L
Adult female: 5-30 IU/L

TO NOTE
1. \(\gamma\) GT helps in differential diagnosis as it is raised only in cholestatic disorders, parallels with ALP levels and not in bone diseases
2. Raised ALP levels with normal \(\gamma\) GT (not found in bone) indicates bone disorder
3. Raised in patients receiving drugs such as phenytoin and phenobarbital, (reflect induction of new enzyme activity).
4. Measurement of \(\gamma\) GT can give an indication of hepatocellular enzyme induction due to drugs or alcohol
5. Isolated elevation or disproportionate elevation of \(\gamma\) GT compared to other liver enzymes indicates alcohol abuse
6. Alcohol might increase \(\gamma\) GT production by inducing hepatic microsomal production or it might cause the leakage of \(\gamma\) GT from hepatocytes.

Lactate Dehydrogenase (LDH)
LDH is normally present in heart, liver, kidney, muscles, brain, blood cells and lungs. It exists in five isoenzyme forms with combination of H and M chains, each isoenzyme fraction being specific to different tissues
1. When LDH isoenzyme spill into blood, it indicates damaged or diseased tissue
2. Each isoenzyme is found in different concentrations in different tissues
3. They differ slightly in their structure with different electrophoretic mobility.
4. LDH-1 (H4) is found primarily in heart muscle and red blood cells.
5. LDH-2 (H3M1) is concentrated in white blood cells.
6. LDH-3 (H2M2) is highest in the lung.
7. LDH-4 (H1M3) is highest in the kidney, liver, placenta and pancreas.
8. LDH-5 (M4) is highest in skeletal muscle.

LDH is involved in the reversible conversion of Pyruvate to Lactate

NORMAL LEVELS
120 -- 240U/L
Normal ratios of different LDH fractions in blood
1. LDH-1: 17% to 27% of total LDH
2. LDH-2: 27% to 37% of total LDH
3. LDH-3: 18% to 25% of total LDH
4. LDH-4: 3% to 8% of total LDH
5. LDH-5: 0% to 5% of total LDH
6. Normally LDH-1 is less than LDH-2 and LDH-5 is less than LDH-4
7. Affinity electrophoresis using polyacrylamide gel is used in estimation of isoenzymes of LDH

TO NOTE
1. Increased LDH isoenzyme fraction level indicates damaged or diseased tissue which along with clinical symptoms and other parameters aids in differential diagnosis.
2. Haemolysed sample gives erroneously high values, as LDH levels are 100 times more in RBC
3. High LDH levels is observed in cancer due to increase in anaerobic glycolysis in tumors
4. Normally LDH-1 is less than LDH-2 but in MI the pattern is reversed showing raised LDH-1 as compared to LDH-2 (Flipped pattern)
5. The tissue source of elevated LDH in serum, can be determined by identifying the individual isoenzyme present in excess by electrophoresis

Group IV Tests.
Group IV Tests includes Immunology blood tests, USG, CT scan, MRI, cholecystography, Liver biopsy

IMMUNOLOGY BLOOD TESTS
1. Immunology blood tests includes detection of HBsAg, HBcAg-IgM, HCV etc
   They are detected by using visual immunochromatographic methods based on the principle of Antigen-Antibody reaction by using the strips supplied by one of the companies mentioned Bionike, Inc ltd., Abbott Laboratories, j.Mitra & Co. Ltd

Virus and antibodies to virus.
Various viral infections can cause inflammation of the liver (hepatitis) and formation of ANA Tests include detection of Hepatitis A virus and Hepatitis B virus.

Auto-antibodies.
Auto-antibodies are antibodies which attack a part of own body. They occur in autoimmune disorders.
An autoantibody is an antibody, a type of protein produced by the immune system that is directed against one or more of the individual's own proteins.

The most common autoimmune disorders are:
1. Primary biliary cirrhosis associated with anti-mitochondrial antibodies
2. Autoimmune hepatitis associated with smooth muscle antibodies
3. Primary sclerosing cholangitis associated with antinuclear cytoplasmic antibodies

TO NOTE
1. Liver function tests (LFT) are often included as a baseline investigation and screening tool
2. Choice of Biochemical Tests to detect Liver disorders is very Important
3. Serial use of LFTs is of most value in following the progress or resolution of liver disease
4. As the liver performs a variety of function, no single test is sufficient to provide complete estimate of functions of liver.
5. LFT reports are not specific and sensitive only to liver disorders(discussed under limitations of LFT)
6. Difficulty in interpreting the LFT reports occurs sometimes as clinical condition of the patient do not tally with the report
7. It is difficult to localize and diagnose from a single abnormal value in LFT, however combinations of tests and enzyme assays aids in differential diagnosis to determine origin of the tissue
8. ALT and AST are used as markers for hepatocellular diseases
9. ALP and γ GT are used as markers for cholestasis diseases
10. γ GT helps in differential diagnosis of cholestatic disorders from bone diseases
11. Raised ALP and γ GT are used as markers for cholestasis diseases.
12. Raised ALP with Normal γ GT indicates bone disorder
13. Summation of symptoms, LFT reports, drug and alcohol abuse, hyperlipedemia, radiological imaging along with Clinical findings helps in proper diagnosis of liver disorders
Differential Diagnosis Of Jaundice

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>HEMOLYTIC JAUNDICE</th>
<th>HEPATIC JAUNDICE</th>
<th>OBSTRUCTIVE JAUNDICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Total Bilirubin</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Direct bilirubin (conjugated)</td>
<td>Normal</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Indirect bilirubin (unconjugated)</td>
<td>Increased</td>
<td>Increased</td>
<td>Absent</td>
</tr>
<tr>
<td>Urine Bile pigments and bile salts</td>
<td>Absent</td>
<td>Increased</td>
<td>Markedly increased</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>Markedly Increased</td>
<td>Normal/Increased</td>
<td>Absent</td>
</tr>
<tr>
<td>Color of Stools</td>
<td>Normal</td>
<td>Normal/Pale</td>
<td>Clay color</td>
</tr>
<tr>
<td>Albumin</td>
<td>Normal</td>
<td>Decreased</td>
<td>Normal</td>
</tr>
<tr>
<td>AST</td>
<td>Normal</td>
<td>Increased</td>
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</tr>
<tr>
<td>ALT</td>
<td>Normal</td>
<td>Markedly Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>ALP</td>
<td>Normal</td>
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</tr>
<tr>
<td>γ GT</td>
<td>Normal</td>
<td>Normal/ Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>Normal</td>
<td>Prolonged</td>
<td>Prolonged</td>
</tr>
</tbody>
</table>

Table 1.2: Findings of different biochemical parameters in jaundice

Limitations of LFT

LFT have certain advantages as well as limitations at the same time.
1. LFT lack specificity and sensitivity and are not specific for any particular disease
2. LFTs are not specific to specific systems or disease processes, yet abnormalities may indicate significant or serious disease.
3. Single abnormalities in LFTs are difficult to localize and diagnose however, the pattern of abnormalities in liver function tests helps to determine the origin of the tissue.
4. Except serum bilirubin levels, the other tests are not specific for liver diseases. Many other parameters may be elevated in other pathological conditions outside the liver.
5. Thus, it is important to view LFT values keeping the clinical profile of the patient in mind.
6. The pattern of abnormalities in LFT helps to determine tissue origin of the disease
7. Liver enzymes may be elevated, even if there is no damage to the liver

Examples for limitations of the liver function tests

LACK SPECIFICITY:
Decrease in serum Albumin levels
- Decrease in serum Albumin is not only present in liver disorders
  1. Serum albumin levels may be decreased in malnutrition, chronic disease and in renal disorders like nephrotic syndrome.

Elevations of Aminotransferases (AST and ALT)
- Increased levels of Aminotransferases are not only found in liver disorders
  1. Aminotransferases may be raised in both cardiac diseases and hepatic diseases
  2. ALT is usually considered to be more specifically related to liver problems.
  3. AST can also be released in heart or skeletal muscle damage
  4. Both AST and ALT are also found to be raised in Paracetamol overdose
  5. They are raised by intake of Phenothiazines, Chlorpromazine, Barbiturates, Tetracycline, Isoniazid, Morphine, Codeine etc

Elevations of Alkaline phosphatase (ALP)
- Increased levels of Alkaline phosphatase is not only found in liver disorder
1. Raised levels of ALP are also seen in bone disorders like osteomalacia, Paget's disease, rickets, bone metastasis, hyperparathyroidism
2. Raised in adolescence (increased bone turnover)
3. Raised in the third trimester of pregnancy (produced by the placenta).
4. D). Elevations of γ Glutamyl Transpeptidase (γ GT)
5. γ GT levels are increased in chronic alcoholics apart from liver disorders

**Elevations of LDH**

Raised LDH is not only found in liver disorder
1. Increased values is also seen in cardiac disorders, skeletal disorders, lung disorders
2. Raised LDH levels is observed in cancer due to increase in anaerobic glycolysis in tumors

**B. Lack sensitivity:**
1. The LFT parameters may be normal in certain liver diseases like cirrhosis, non-cirrhotic portal fibrosis, congenital hepatic fibrosis etc

**TO NOTE**
2. It is difficult to localize and diagnose the liver disorders by single abnormality of liver function tests however, the pattern of abnormalities helps to determine origin of the tissue.
3. Clinicians are faced with reports that do not tally with the clinical condition of the patient and they find difficulty in interpreting the LFT
4. Treatment of jaundice typically requires selecting proper panel tests of LFT, interpret the values to localize the tissue for diagnosis along with clinical symptoms for selection of suitable treatment options and modalities
5. Thus, it is important to view LFT report, keeping the clinical profile of the patient in mind as LFT have certain advantages and limitations at the same time as explained
6. Levels of various parameters in serum, urine and stools along with the clinical picture, medication, drug history and the presence of any current or recent symptoms helps in differential diagnosis of Jaundice into non-hepatic, hepatocellular, cirrhosis, cholestatic pattern or other forms of abnormality
7. Treatment would then target the cause, rather than the jaundice itself.
8. Hepatic elastography is a noninvasive imaging technique used to determine the degree of fibrosis of the liver

**JAUNDICE IN NEWBORNS**

**Physiological jaundice.**
1. Jaundice is the most common condition that requires medical attention in newborns.
2. Jaundice occurs in as many as 60% of all normal newborns within the first week of life
3. It is more commonly due to the normal physiological inability of the newborn infant to process bilirubin adequately resulting in transient Hyperbilirubinemia
4. The yellow coloration of the skin and sclera in newborns is the result of accumulation of unconjugated bilirubin
5. In most infants with physiologic jaundice, bilirubin concentrations do not rise to a point that requires treatment.
6. Hyperbilirubinemia is due to the combined effects of increased RBC turnover and a transient deficit of enzyme glucuronic transpeptidase, which conjugates insoluble bilirubin with glucuronic acid forming water soluble bilirubin diglucuronide which is excreted along the bile.
7. Nonpathologic jaundice is referred to as physiologic jaundice of the newborn

**Causes**
Increased haemolysis, immature hepatic system for the uptake, conjugation and secretion of bilirubin

**Pathological jaundice**

**kernicterus**

In some infants due to some pathological condition / inborn metabolic errors, serum unconjugated bilirubin levels may raise beyond 20 mg/dl which can cross blood-brain barrier causing acute and chronic bilirubin encephalopathy (kernicterus)
1. It may be either due to excess haemolysis with increased bilirubin production or decreased bilirubin conjugation and its excretion.
2. Unconjugated bilirubin is neurotoxic, can cause kernicterus and death in newborns.
3. kernicterus results in a lifelong neurologic sequelae in infants who survive that put the infant at risk of mental retardation

**Causes-**
Increased serum unconjugated bilirubin in newborn may be either due to—

1. Increased hemolysis
2. Immature liver
3. Deficiency of conjugating enzyme
4. Defect in conjugation
5. Metabolic disorders
6. Treatment

Treatment for severe jaundice in new born is essential in order to avoid serious manifestations of kernicterus.

**Effective treatments includes-**
1. Phototherapy
2. Phenobarbital drug therapy
3. Exchange Transfusion.

**GENETIC DEFECTS**
Intrinsic liver disease due to Genetic defects

**Crigler–Najjar Syndrome**
Crigler–Najjar syndrome is a rare inherited autosomal recessive disorder affecting the bilirubin metabolism. The disorder results in nonhemolytic jaundice, which results in high levels of unconjugated bilirubin which often leads to brain damage in infants.

**Crigler–Najjar Syndrome Type I**
1. This is a very rare autosomal recessive Inherited disease
2. Consanguinity increases the risk of this condition
3. Jaundice appears in the first days of life and persists thereafter and the child hardly lives beyond two years
4. Defect in enzyme UDP glucurononyl transferase affects the conjugation resulting in increase in unconjugated bilirubin usually above 20 mg/dL

**Crigler–Najjar Syndrome Type II**
1. Crigler–Najjar syndrome II is less dangerous than Type I as there is defect in the addition of second glucurononyl group
2. Bilirubin levels are generally less than 20 mg/dL and some cases are only detected later in life.
3. Because of lower serum bilirubin levels, kernicterus is rare in type II.

**Gilbert's Syndrome**
1. Gilbert's syndrome has **no serious** consequences and usually asymptomatic as there is mild jaundice(1.2 to 4 mg/dl)
2. This type of jaundice may be either due to fault in bilirubin uptake, conjugation or excretion
3. Mild increase in serum bilirubin may appear under conditions like exertion, stress, fasting and infections
4. Several analyses have found a significantly decreased risk of coronary artery disease (CAD) in individuals with Gilbert's syndrome

**Dubin-Johnson Syndrome**
1. Dubin-Johnson syndrome is rare autosomal recessive inherited disorder
2. Its inheritance is autosomal recessive and the prognosis is good. It is a relapsing, benign disorder of bilirubin metabolism
3. It is characterized with conjugated hyperbilirubinemia usually in the range of 2 to 5-mg/dL with normal liver enzymes.