Review Article

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Evaluation and interpretation of biomarkers of liver diseases

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Abstract:

Liver performs a variety of different biochemical, synthetic and excretory functions so no single biochemical test can detect the global functions of liver. All the laboratories usually employ a battery of tests for initial detection and management of liver diseases known as liver function tests. Often, liver disease is clinically silent until late in its course and clinicians are faced with reports that do not tally with the clinical condition of the patient and they face difficulty in interpreting the liver function test. For this reason, biochemical laboratory tests are of immense value in diagnosis and monitoring of liver diseases. An attempt is being made to study liver function test and simplify their interpretation.

Key words:5' nucleotidase; α-fetoprotein; Alanine amino transferase;Alkaline phosphatase; Aspartate amino transferase; Bilirubin;Ceruloplasmin; Gamma glutamyl transferase;Liver function test;Ratio of aminotransferases;

Liver is a versatile organ, which is involved in metabolism and independently in many other biochemical functions.

[A.] **Metabolic functions:** Liver is the key organ and the principal site where the metabolism of carbohydrates, lipids and proteins take place[1-6].

i) **Carbohydrate Metabolism:** Glucose may be metabolized though glycolysis and then to citric acid cycle and oxidative phosphorylation to yield energy, if the cells are in need of ATP. If ATP is not required, then glucose can be stored as glycogen within the liver or it can be converted into more stable storage form as triglycerides.

ii) **Lipid Metabolism:** Fatty acids will be catabolized to release acetyl-CoA. It may be used in the TCA cycle and ETC a source of carbon for fatty acid and cholesterol synthesis in healthy individuals. A small portion of acetyl- CoA is converted to ketone bodies (acetone, acetoacetic acid and beta hydroxybutyric acid). Dietary lipids are repacked and secreted into the systemic circulation as lipoproteins. The protein pars of the lipoproteins, apoproteins are synthesized by the liver only. Hence, the liver has an important role in the distribution of lipids in the body.

iii) **Protein Metabolism:** Proteins are break down in the intestine and absorbed as a amino acids, which then reach liver by portal vein. There, they may be utilized to form proteins of different kinds. Some of them are produced only in the liver, like albumin, α and β globulins and coagulation factors I, II, V, VII, IX & X. Several proteins of acute phase reactants are produced in the liver for example C-reactive protein.

iv) **Bilirubin Metabolism:** The heme present in the hemoglobin and other proteins/enzymes (cytochromes) are eliminated only through liver. The lysis of red blood cells releases hemoglobin, which splits to release globin and heme. The heme part is catabolized by microsomal heme oxygenase system of reticuloendothelial system produce bilirubin. The bilirubin (unconjugated) thus formed is hydrophobic in nature hence it is transported in the blood by binding with albumin to reach the liver. In the liver, it is conjugated with glucuronic acid to form hydrophilic conjugated bilirubin and is excreted in bile into the intestine. Bacterial acion (deconjugation and reduction) forms bilinogen (stercobilinogen, mesobilinogen and urobilinogen). About 20% of the urobilinogen is reabsorbed daily from the intestine to enter enterohepatic circulation to get re excreted into the intestinal lumen (enterohepatic circulation) [2,7].

A small fraction of urobilinogen enters the systemic circulation and gets filtered at the glomerulus and excreted in the urine (Figure-1).

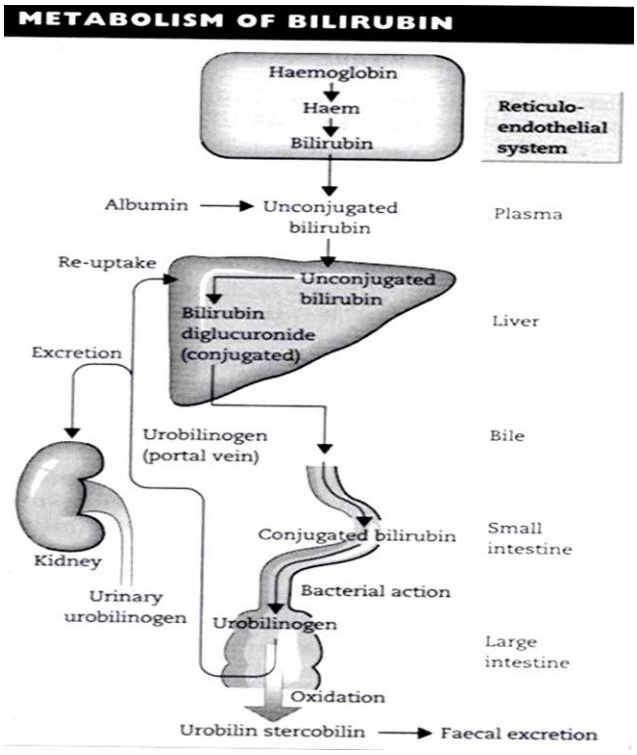


Figure 1: Metabolism of Bilirubin

[B] Secretory functions: Liver is responsible for the formation and secretion of bile in the intestine. Bile pigments –bilirubin formed from heme catabolism is conjugated in liver cells and secreted in the bile [8,9].

[C]Excretory functions: Substances detoxified by the liver are excreted through bile. About 3 liters of bile is produced daily and the rest is reabsorbed and out of this 1liter is excreted and the rest is reabsorbed and circulated in the enterohepatic circulation. The bile contains bile salts, conjugated bilirubin, phospholipids and hormones. Major route of cholesterol excretion is through bile. The bile reaching the intestine facilitates the digestion and absorption of lipids and fat-soluble vitamins [10,11].

[D] Detoxification functions: Liver plays a central role in various detoxifying reactions [7-9].

i) Exogenous Substances: Toxic substances entering from gut and parental route are mainly detoxified in the liver by different reactions like hydrolysis, hydroxylation, oxidation, carboxylation, reduction and demethylation. The detoxified products are more water soluble and thus easily exctreted in urine. The cytochromes P450 enzyme system of hepatocyte is mainly concerned with drug metabolism; conversion of drugs into more soluble forms, which in due course conjugate with compounds like gycine, glucuronic acid and glutathione and finally excreted either in urine or through bile.

ii) Endogenous Substances: Disposal of bilirubin is already above discussed (Figure-1). Ammonia produced from amino acid catabolism is detoxified the liver to form less toxic urea. The key enzymes of urea cycle are located entirely in liver [14,15].

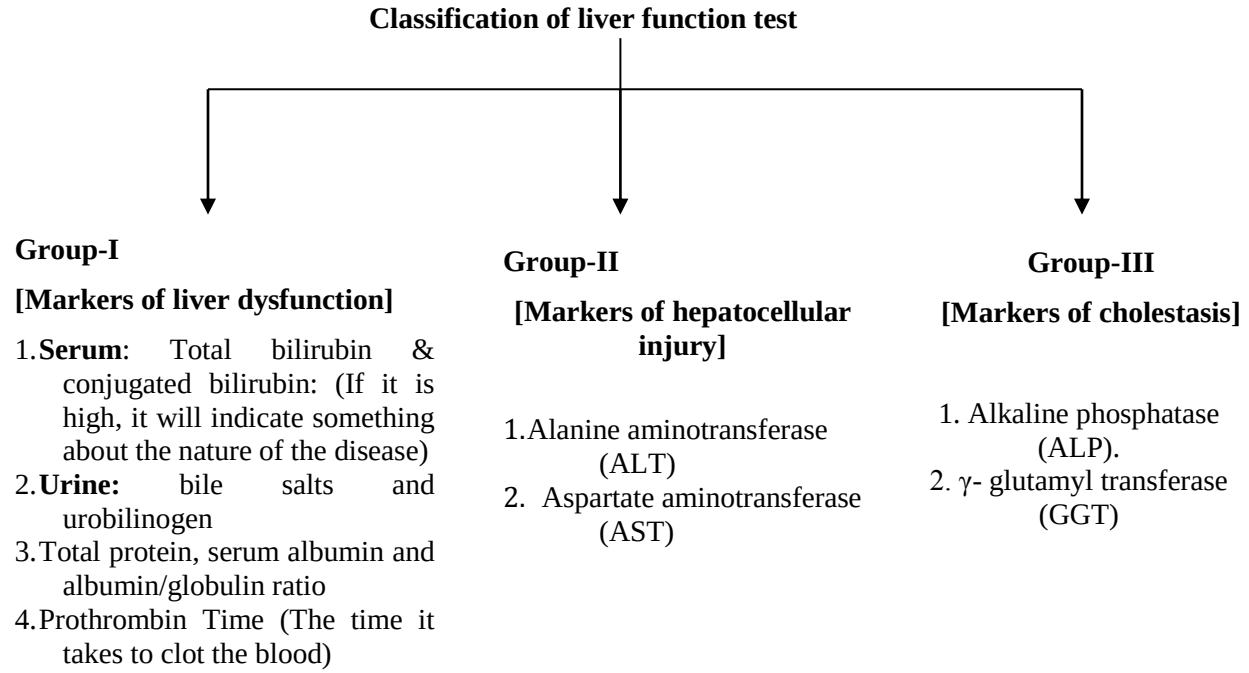
[E] Storage functions: Liver stores glucose in the form of glycogen. It also stores Vitamins K, A, D, E, B12 [3-5,10,11].

The liver has an indispensible role in the intermediately and energetic metabolism of carbohydrates, lipids and proteins. It provides inactivation and excretion of many endogenous / exogenous substances as well as synthesis of plasma proteins and blood clotting factors as discussed above. Diseases of the liver alter many biochemical parameters in the serum, in turn the assessment of these biochemical parameters are important for the diagnosis of liver diseases. Morphologically, About 72% of the liver tissue is hepatocytes, the Kupffer cells, endothelium and adipose cells together form another 8 %; bile tracts contribute 1% and the rest is extracellular fluid. In an acute liver damage mainly hepatocytes are affected, in other diseases, impairment of bile flow leading to predominant damage to bile capillary cells may prevail [12].

Numerous laboratory investigations have been proposed in the assessment of liver diseases. From among these host of tests, the following battery of blood tests like total, conjugated & unconjugated bilirubin, total and differential proteins & albumin: globulin ratio, and certain enzyme assays such as Aminotransferases, alkaline phosphatase and γ glutamyl transferase. Urine tests for bilirubin and its metabolites, prothrombin time and prothrombin index have become widely known as standard liver function tests (LFTs).

The second generation LFT attempt to improve on this blood battery of tests and to gain a genuine measurement of liver functions like quantitative assessment of functional hepatic mass. These include the capacity of the liver to eliminate exogenous compounds such as aminopyrine or caffeine or endogenous compounds like bile acids, which have gained much importance recently. However such investigations are not yet routinely. In this article, only standard LFTs which are routinely done and possible in any standard laboratory are discussed and simplify their interpretation.

Liver function test are classified broadly into three groups as follows;



Group-1: Markers of liver dysfunction:

[A] Measurement of serum bilirubin (test of excretory function of liver): Bilirubin is an endogenous anion formed by the catabolism of heme. The measurement of bilirubin as well as detection of bilirubin and urobilinogen in urine is important tests of liver function. The classification of bilirubin into direct and indirect bilirubin is based on the original Van der Bergh method of measuring bilirubin. According to Van der Bergh, bilirubin in serum forms a purple compound that is azo-bilirubin, where bilirubin in serum is allowed to react with solution of Van der Bergh's diazo-reagent. Conjugated bilirubin is water soluble and unconjugated bilirubin is not soluble in water and it requires solubilizer that is alcohol. Hence when the reaction is carried out in alcohol then total bilirubin is estimated. Unconjugated bilirubin is estimated by subtracting conjugated bilirubin from total bilirubin[13-15].

Serum Bilirubin levels:

1. Normal: 0.2 to 0.8mg/dl
2. Indirect/Unconjugated/ Free: 0.2 to 0.7 mg/dl
3. Direct/conjugated: 0.1 to 0.4mg/dl

Latent Jaundice: Above 1mg/dl (patient does not present with jaundice (subclinical jaundice)

Jaundice: Above 2mg/dl (High bilirubin levels are observed in gallstones, acute and chronic hepatitis)[16].

Bilirubin in body is a careful balance between production and removal of the pigment in body. Hyperbilirubinemia seen in acute viral hepatitis is directly proportional to the degree of histological injury of hepatocytes and the longer course of the disease.

Hyperbilirubinemia: It results from overproduction /impaired uptake, conjugation or excretion / regurgitation of unconjugated or conjugated bilirubin from Hepatocytes to bile ducts.

Increased unconjugated bilirubin: This results from overproduction/impaired uptake of conjugation.

Increased conjugated bilirubin: Impaired intrahepatic excretion / regurgitation of unconjugated or conjugated bilirubin from hepatocytes of bile ducts. Serum bilirubin could be lowered by drugs like salicylates, sulphonamides, free fatty acids which displace bilirubin from its attachment to plasma albumin. On the contrary it could be elevated if the serum albumin increases and the bilirubin may shift from tissue sites to circulation. Bilirubin may be of prognostic value in conditions like fulminant hepatic failure where deep jaundice is associated with increased mortality[15,16].

Hyperbilirubinemia and Hemolysis: Bilirubin itself is not soluble in water and is bound to albumin and thus does not appear in urine. Hemolysis with overproduction of bilirubin and concomitant reduced GFR causes decreased excretion and can lead to high bilirubin levels. Bilirubin levels in excess of 25 mg/ dl may be seen in hemolysis in association with liver disease. Other causes of extreme hyperbilirubinemia include severe parenchymal disease, septicemia and renal failure[15-17].

2. Urine bilirubin

The presence of urine bilirubin indicates hepatobiliary disease.

a. In all cases of jaundice, urine should be examined for the presence of bile pigments (bilirubin), bile salts and urobilinogen.

b.Only conjugated bilirubin is soluble in water and is excreted in urine. Hence, in pre- hepatic jaundice, when the unconjugated bilirubin is increased in blood, it does not appear in urine; hence called acholuric jaundice.

c.But in obstructive jaundice, conjugation of bilirubin is takes place, which cannot be excreted through the normal passage and so, it is regurgitated back into blood stream. This is then excreted through urine. So, in obstructive jaundice, urine contains bilirubin (Choluric jaundice).

Urobilinogen: Most of the urobilinogen is metabolized in the large intestine (into strecobilin and excreted via feces) and small fraction is excreted in urine (less than 4mg/day). An increase in the urobilinogen in urine is a sensitive indicator of hepatocellular dysfunction.

a. In cases of obstruction, bile is not reaching the intestine and so urobilinogen may be decreased or absent in urine.

b.In hepatocelluar jaundice, urobilinogen is initially elevated, then decreases or disappears when the obstructive stage sets in and reappears when obstruction is cleared.

c.Urobilinogen is absent in urine, when there is obstruction to bile flow. The first indication of recovery is the reappearance of urobilinogen in urine.

d.In hemolytic anemias, urobilinogen is increased.

e.Bilirubinis detected by Fouchest's test and urobilinogen by Ehrlich's test.

Bile Salts: Normal bile salts (sodium slats of taurocholic acid and glycocholic acid are present in the bile, but are not seen in urine. Bile salts in urine are detected by Hay's sulfur test. Positive Hay's test indicates the obstruction in the biliary passages causing leakage of bile salts into the systemic circulation leading to its excretion in urine. Obstruction can occur in obstructive jaundice and also in hepatic jaundice due to obstruction of micro biliary channels caused by inflammation.

[B] Measurement of Serum proteins (test of synthetic function of liver):

Almost all the plasma proteins except immunoglobulins are synthesized by liver. The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of α and β globulins[2,6,8,9].

1. Albumin: Serum albumin is the most important protein synthesized by the liver. The synthesis of albumin reflects the extent of functioning of liver cell mass. The extent of decrease in serum albumin level is directly proportional to the extent of liver damage. The half life of serum albumin is as long as 20days, hence serum albumin levels is not reliable indicator of hepatic protein synthesis in acute liver diseases whereas in all chronic diseases of liver, the albumin level is decreased.

A normal serum level of albumin is 3.5 to 5.0g/dL. Corticosteroids and thyroid hormone stimulate albumin synthesis by increasing the concentration of albumin mRNA and tRNA in hepatocytes[18].

The serum albumin levels tend to be normal in diseases like acute viral hepatitis, drug related hepatotoxicity and obstructive jaundice. Albumin levels below 3g/dl in hepatitis should raise the suspicion of chronic liver disease like cirrhosis which usually reflects decreased albumin synthesis. In ascites there may be normal synthesis but the levels may appear reduced because of increased volume of distribution. Hypoalbuminemia is not specific for liver disease and may occur in protein malnutrition, nephrotic syndrome and chronic protein losing enteropathies[15,19,20].

2. Globulin: They constitute immunoglobulin's produced by B lymphocytes as well as α and β globulins synthesized mainly by hepatocytes. Gamma globulins in the serum are increased in chronic liver diseases like chronic active hepatitis, and cirrhosis. In cirrhosis, antibodies against intestinal bacteria are seen, since the cirrhotic liver cannot clear the bacteria reaching through circulation. IgG is increased in autoimmune hepatitis, IgM is increased in primary biliary cirrhosis and IgA is increased in alcoholic liver disease[21].

Albumin to Globulin (A/G) ratio: Normal A/G ratio: 1.2/1- 1.5/1.2. Globulin levels increase in hypoalbuminemia as a compensatory mechanism to maintain serum protein, finally result in decreased A/G ratio.

3. Prothrombin Time (PT): Clotting is the end result of a complex series of enzymatic reactions that involve at least 13 factors. The liver is the major site of synthesis of 11 blood coagulation proteins (fibrinogen, prothrombin, labile factor, stable factor, Christmas factor, Stuart power factor, Prekallikrein and high molecular weight Kininogen. The estimation of prothrombin is a useful indicator of liver function. The half life of prothrombin is 6hours only, therefore PT indicates the present function of the liver. The results of this test may be expressed in sec or as a ratio of the plasma prothrombin time to control plasma time. Normal control usually is in the range of 9-11 seconds. A prolongation of more than 2 seconds is considered abnormal. The prolonged PT is not specific for liver diseases and is seen in various deficiencies of coagulation factors, DIC, and ingestion of certain drugs. In acute and chronic hepatocellular disease the PT may serve as a prognostic indicator. In acute hepatocellular disease worsening of PT suggests an increased likelihood of acute hepatic failure[15].

The PT is a predictor of outcome in cases of acetoaminophen over dosage and acute alcoholic hepatitis. Prolongation of PT is also suggestive of poor long-term outcome in chronic liver disease. If the PT returns to normal or improves by at least 30% within 24 hr of a single parenteral injection of vitamin K1 (5-10 mg), it may be surmised that parenchymal function is good and that hypovitaminosis K was responsible for the original prolongation of PT. Patients with parenchymal disease by contrast will show only minimal improvement. Most patients with extra hepatic obstruction like EHBA would respond promptly to a single injection of vitamin K1. The PT is particularly important in the management of patients with liver disease. It is important to perform before procedures like liver biopsy and kidney biopsy and it permits an assessment of the tendency to bleed. In many centers the International normalized ratio (INR) is done in place of PT[15,16].

4. α- Fetoprotein (AFP): AFP is a normal component of fetal blood. It disappears after birth within a few weeks. It is a tumor marker[23,24].

-Mild elevation in AFP is suggestive of chronic hepatitis or cirrhosis.

-A drastic increase is seen in hepatocelular carcinoma, germ cell tumors and teratoma of ovary.

-Elevation is seen in cases of fetal open neural tube defects and also in cases with multiple fetuses or fetal death.

-Low AFT is seen in maternal serum in cases of fetal Down syndrome.

Immune assay is employed to test AFP. Reference limits are upto 1year ≤30ng/mL and in adults (male and non pregnant females ≤15ng/mL[25].

5. Ceruloplasmin (Cp): Ceruloplasmin is synthesized in the liver and is an acute phase protein. It binds with the copper and serves as a major carrier for copper in the blood. Normal plasma level of Cp is 200 to 600mg/L[26,27].

-The level is elevated in infections, rheumatoid arthritis, pregnancy, non Wilson liver disease and obstructive jaundice.

-Low levels may also be seen in neonates, menke's disease, kwashiorkor, marasmus, protein losing enteropathy, copper deficiency and aceruloplasminemia. In Wilson's disease ceruloplasmin level is depressed. Decreased rate of synthesis of the ceruloplasmin is responsible for copper accumulation in liver because of copper transport defect in golgi apparatus, since ATP7B is affected[28,29].

-Serum ceruloplasmin levels were elevated in the chronic active liver disease (CALD) but lowered in the Wilson's disease (WD). Hence it is the most reliable routine screening test to differentiate between CALD and WD[30].

6. Transthyretin (Pre-Albumin): Transthyretin is synthesized by the liver. Its major functions are transport of thyroxine and triiodothyronine. The half life of pre- albumin is only 2days. Hence, it is a useful biochemical assay to assess the hepatic function early in the course of liver disorders. The serum transthyetin level is 0.2-0.3g/L. The levels of pre-albumin falls in liver disease presumably due to reduced synthesis due to short half life. The determination of pre-albumin has been consider particularly useful in drug induced hepatotoxicity.

7. α-1 Antitrypsin (AAT): AAT is a acute phase reactant and is synthesized and secreted by the liver. It is an inhibitor of serum proteases like elastase and collagenaase. The various alleles coded are M, F, S, Z and null forms. PiZZ homozygotes are associated with neonatal hepatitis. Cirrhosis in adults has been found with ZZ, MZ, SZ and FZ phenotypes.

Reference limits of AAT is 1-1.6g/L

-Low levels of AAT are associated with neonatal cholestasis, progressive juvenile cirrhosis in children and micronodular cirrhosis in adults. Low levels are also seen in panlobular emphysema.

-AAT levels increased inacute trauma, infections or after estrogen therapy and I many malignancies[14].

8. Hepatogloblin: Hepatogloblin (acute phase reactant) is synthesized in the liver. It transports free hemoglobin in the plasma to reticuloendothelial system. The free Hb (not bound to haptogloin) is freely filtered at the glomerulus and get precipitated in the tubules leading to damage to kidneys. Hepatogloblin bound Hb complex being large can not be filtered at the glomerulus and thus retained in the circulation. Hepatogloblin bound Hb complex is degraded by the reticuloendothelial system leading to rapid depletion of hepatoglobin from circulation in cases of exaggerated hemolysis.

-Low levels of hepatoglobin are seen with severe hepatocellular liver disease (deficient synthesis)and in hemolytic disease (increased rate of degradation).

-Being acute phase reactant, hepatogloblin's levels are increased in inflammatory processes, trauma, infections and myocardial infarction.

A reference limit of hepatoglobin in serum is 30-200mg/Dl.

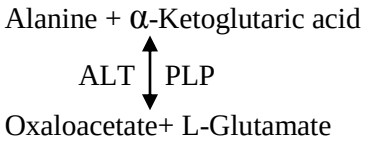
The turnover rates of hepatoglobin and transferrin are lesser than albumin; hence they are useful to identify the recent changes in liver functions.

Group-II: Markers of hepatocellular injury

The aminotransferases (formerly transaminases) are the most frequently utilized and specific indicators of hepatocellular necrosis. Aminotransferases are enzymes that catalyze reversible transfer of the amino group from an amino acid to a ketoacid. Pyridoxal-5'-phosphate (PLP) serves as a cofactor. In clinical biochemistry, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most significant. AST, formerly serum glutamate oxaloacetic transaminase (SGOT) and ALT, formerly serum glutamic pyruvate transaminase (SGPT) catalyze the transfer of the α- amino acids of aspartate and alanine respectively to the α- keto group of ketoglutaric acid[30].

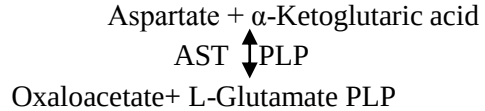
ALT catalyzes a reversible transfer of amino group from alanine to α- keto group of α-ketoglutaric acid. ALT is present mostly in the liver; its activity in other organs (skeletal muscle, myocardium, etc.) is much lower. Unlike AST it localizes only to the cytosol. Estimation of ALT is a sensitive and relatively specific test for hepatocyte damage. Its activity in serum rises even in a small damage of the liver cell, caused by increased permeability of the cell membrane. In inflammation of the liver (viral hepatitis), for instance, elevation of ALT is the earliest indicator that hepatocyte cell membrane integrity is compromised. Repeated ALT estimation is suitable for monitoring course of the disease[32,33].

Reference values of serum ALT (S-ALT): Men up to 0.80 μkat/L and in women up to 0.60 μkat/L.



ALT catalyzes a reversible transfer of amino group from aspartate to α- keto group of α-ketoglutaric acid. AST occurs in numerous organs: liver, heart, skeletal muscle, kidney, pancreas, and red blood cells. It exists in two isoenzymes: mitochondrial (about 70%), and cytosolic (about 30%). Cytosolic fraction is readily released into circulation due to mild alterations of hepatocyte cell membrane permeability. In contrast, the mitochondrial fraction is released only after destruction (necrosis) of the hepatocyte. Therefore, a high increase of serum AST is a marker of hepatocyte destruction, because both isoenzymes are likely to participate in the increase. Since AST is not specific for the liver tissue, it can be elevated also in damage of skeletal muscle and myocardium. AST in blood rises in acute myocardial infarction (heart stroke) and following heart surgery, but also due to a long lasting strenuous physical exercise. Hemolysis of the sample can cause false positive results of AST estimation, since quite high levels of the enzyme are present in the erythrocytes.

Reference values of serum AST (S-AST): Men up to 0.85 μkat/L and in women up to 0.60 μkat/L.



Their activity in serum at any moment reflects the relative rate at which they enter and leave

circulation. Of the numerous methods used for measuring their levels, the most specific method couples the formation of pyruvate and oxaloacetate- the products of the aminotransferase reactions to their enzymatic reduction to lactate and malate. Virtually no aminotransferases are present in the urine or bile and hepatic sinusoids are the primary site for their clearance[32-34].

Mild, moderate and severe elevations of aminotransferases

1. **Severe (> 20 times, 1000 U/L):** The AST and ALT levels are increased to some extent in almost all liver diseases. The highest elevations occur in severe viral hepatitis, drug or toxin induced hepatic necrosis and circulatory shock. Although enzyme levels may reflect the extent of hepatocellular necrosis they do not correlate with eventual outcome. In fact declining AST and ALT may indicate either recovery of poor prognosis in fulminant hepatic failure[14,16].

2. **Moderate (3-20 times):** The AST and ALT are moderately elevated in acute hepatitis, neonatal hepatitis, chronic hepatitis, autoimmune hepatitis, drug induced hepatitis, alcoholic hepatitis and acute biliary tract obstructions. The ALT is usually more frequently increased as compared to AST except in chronic liver disease. In uncomplicated acute viral hepatitis, the very high initial levels approach normal levels within 5 weeks of onset of illness and normal levels are obtained in 8 weeks in 75% of cases. For reasons, which are not, understood AST levels appear disproportionately low in patients with Wilson disease[14,16].

3. **Mild (1-3 times):** These elevations are usually seen in sepsis induced neonatal hepatitis, extrahepatic biliary atresia (EHBA), fatty liver, cirrhosis, non alcoholic steato hepatitis(NASH), drug toxicity, myositis, duchenne muscular dystrophy and even after vigorous exercise.1,4 One third to one half of healthy individuals with an isolated elevation of ALT on repeated testing have been found to be normal[35].

The severity of hepatocyte damage can also be assessed from the AST: ALT ratio, also known as De Ritis Quotient. A value higher than 1 is considered as a sign of unfavorable prognosis. The ratio of AST to ALT is useful in Wilson disease, CLD and alcoholic liver disease and a ratio of more than 2 is usually observed. In NASH, the ratio is less than one in the absence of fibrosis on liver biopsy. In viral hepatitis the ratio is usually less than one. The ratio invariably rises to more than one as cirrhosis develops possibly because of reduced plasma clearance of AST secondary to impaired function of sinusoidal cells. ALT exceeds AST in toxic hepatitis, viral hepatitis, chronic active hepatitis and cholestatic hepatitis[14,16,36].

Some other enzymes of hepatocellular necrosis, which have been found to be useful but are not routinely done in the laboratory. These include glutamate dehydrogenase, isocitrate dehydrogenase, lactate dehydrogenase and sorbitol dehydrogenase.

Group-II: Markers of cholestasis (obstructive liver disease)

1. **Alkaline Phosphatase (ALP):** ALPs are a family of zinc metaloenzymes, with a serine at the active center; they release inorganic phosphate from various organic orthophosphates and are present in nearly all tissues (mucosal epithelia of small intestine, proximal convoluted tubule of kidney, bone, liver and placental). ALP performs transportation in the intestine and calcification in bone. The serum ALP activity is mainly from the liver with 50% contributed by bone [25].

Normal serum ALP is 41 to 133U/L [26]. Average values of ALP vary with age and are relatively high in childhood and puberty and lower in middle age and higher again in old age. Males usually have higher values as compared to females. The levels correlate with person's weight and inversely with the height of person[37].

-Highest levels of ALP (10-12 time of upper limit) occur in cholestatic disorders. Elevations occur as a result of both intrahepatic and extrahepatic obstruction to bile flow and the degree of elevation does not help to distinguish between the two. ALP levels are likely to be very high in EHBA[16]. The mechanism by which ALP reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions] and the other hypothesis is that the damaged liver fails to excrete ALP made in bone, intestine and liver[14-16,39].

-Drastically high levels of ALP (0-25 times upper limit) are seen in bone disease, where osteoblastic activity is enhanced. For example; paget's disease (osteitis deformans), rickets, osteomlacia, osteoblastoma, metastatic carcinoma of bone and hyperparathyroidism.

-Tumours secrete ALP into plasma and there are tumour specific isoenzymes such as Regan, Nagao and Kasahara. Hepatic and bony metastasis can also cause elevated levels of ALP. Other diseases like infiltrative liver diseases, abscesses, granulomatous

liver disease and amyloidosis may cause a rise in ALP[28].

-Mildly elevated levels of ALP may be seen in cirrhosis, hepatitis and congestive cardiac failure[28].

-Low levels of ALP occur in hypothyroidism, pernicious anaemia, zinc deficiency and congenital hypophosphatasia. ALP activity was significantly higher in the third trimester of asymptomatic normal pregnancy showing extra production from placental tissue. ALP levels in hyperemesis gravidarum were 21.5U/L, in pre-eclampsia 14U/L, and 15U/L in haemolysis with low platelet count was seen during symptomatic pregnancy[39-41].

-Transient hyperphosphataemia in infancy is a benign condition characterized by elevated ALP levels of several folds without evidence of liver or bone disease and it returns to normal level by 4 months[42].

-ALP has been found elevated in peripheral arterial disease, independent of other traditional cardiovascular risk factors. Often clinicians are more confused in differentiating liver diseases and bony disorders when they see elevated ALP levels and in such situations measurement of gamma glutamyl transferase assists as it is raised only in cholestatic disorders and not in bone diseases[14,43].

2. γ- Glutamyl Transferase (GGT): GGT is a microsomal enzyme, present in hepatocytes and biliary epithelial cells, renal tubules, pancreas and intestine. It is also present in cell membrane performing transport of peptides into the cell, across the cell membrane and is involved in glutathione metabolism. Serum GGT activity mainly attributed to hepatobiliary system even though it is found in more concentration in renal tissue [25].

γ-glutamyl-cysteinyl-glycine (GSH)⇔γ-glutamyl amino acid

+ Amino Acid or Peptide + Cysteinyl- Glycine

The basic GGT-catalyzed reaction:

The levels of GGT are high in neonates and infants up to 1 year and also increase after 60 year of life. Men have higher values. Children more than 4 year old have serum values of normal adults. The normal range is 0-30IU/L [14,15].

-In acute viral hepatitis the levels of GGT will reach the peak in the second or third week of illness and in some patients GGT remain elevated for 6 weeks [14].

-In liver disease, GGT activity correlates well with ALP levels but rarely the GGT levels may be normal in intra hepatic cholestasis like in some familial intrahepatic cholestasis [44].

-Increased level is seen in about 30% of patients with chronic hepatitis C infection [45]. Other conditions like uncomplicated diabetes mellitus, acute pancreatitis, myocardial infarction, anorexia nervosa, Gullian barre syndrome, hyperthyroidism, obesity and dystrophica myotonica caused elevated levels of GGT [14].

-Elevated serum GGT levels of more than 10 times is observed in alcoholism. It is partly related to structural liver damage, hepatic microsomal enzyme induction or alcoholic pancreatic damage [46].

-GGT can also be an early marker of oxidative stress since serum antioxidant carotenoids namely lycopene, α-carotene, β-carotene, and β-cryptoxanthin are inversely associated with alcohol-induced increase of serum GGT found in moderate and heavy drinkers [47].

-GGT levels may be 2–3 times greater than the upper reference value in more than 50% of the patients with nonalcoholic fatty liver disease [48]. There is a significant positive correlation between serum GGT and triglyceride levels in diabetes and the level decreases with treatment especially when treated with insulin. Whereas serum GGT does not correlate with hepatomegaly in diabetes mellitus [49].

-Serum GGT activity was significantly lower in the second and third trimesters of normal asymptomatic pregnancy. The levels of GGT in hyperemesis gravidarum was 45U/L, in preeclampsia 17U/L, and 35U/L in hemolysis with low platelet count and elevated liver enzymes was found during symptomatic pregnancy[40,41].

-Clinicians are faced with a dilemma when they see elevated ALP levels and are unable to differentiate between liver diseases and bony disorders and in such situations measurement of GGT helps as it is raised only in Cholestatic disorders and not in bone diseases [14].

4. 5' Nucleotidase (NTP): NTP is a glycoprotein generally disseminated throughout the tissues of the body localised in cytoplasmic membrane catalyzing release of inorganic phosphate from nucleoside-5-phosphates.

The normal range of NTP is 0 to 15U/L[25].

-Raised levels of NTP activity were found in patients with obstructive jaundice, parenchymal liver disease, hepatic metastases and bone disease[14]. NTP is precise marker of early hepatic primary or secondary

tumours. ALP levels also increased in conjugation with NTP showing intra or extra hepatic obstruction due to malignancy[50].

-Elevation of NTP is found in acute infective hepatitis and also in chronic hepatitis. In acute hepatitis elevation of NTP activity is more in relation with chronic hepatitis and it is attributed to shedding of plasma membrane with ecto NTP activity due to cell damage, or leakage of bile containing high NTP activity[51,52].

-Elevation in serum NTP activity is not noticed in childhood and pregnancy as in the case of ALP. Hence estimation is more specific for obstructive liver disease.

5.Carbohydrate-deficient transferrin (CDT): Transferrin is a glycoprotein that contains in its molecule usually from four to six moieties of sialic acid. In case of chronic alcohol abuse (60 g of alcohol daily for period at least two to three weeks) the fraction of transferrin lacking the sialic acid moieties – the CDT – increases. If the fraction of CDT exceeds 6 % of total transferrin, it indicates a chronic alcohol abuse. The CDT remains elevated for about 2 weeks following discontinuation of alcohol intake. Recently, GGT was considered as the best marker of one of the commonest causes of liver disease, the chronic alcohol abuse. Nowadays, however, a new parameter has appeared that is CDT.

Conclusion

Liver performs different kinds of biochemical functions, so no single biochemical test can detect the global functions of liver. Many serious liver diseases may be associated with normal and abnormal levels might be found in asymptomatic healthy individuals. The battery of liver tests helps in establishing the diagnosis of liver disease. The assessment of enzyme abnormalities along with some other parameter, the rate of change and the nature of the course of alteration or follow up of 6 months to 2 years helps in the diagnosis of the disease. The pattern of enzyme abnormality, interpreted in the context of the patient's symptoms can aid in directing the subsequent diagnosis.

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