LIVER FUNCTION TEST – A REVIEW

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LIVER PHYSIOLOGY

The liver is the largest organ of the human body, weighs approximately 1500 g, and is located in the upper right corner of the abdomen.

The major blood vessels, portal vein and hepatic artery, lymphatics, nerves and hepatic bile duct communicate with the liver at a common site, the hilus. From the hilus, they branch and rebranch within the liver to form a system that travels together in a conduit structure, the portal canal. From this portal canal, after numerous branching, the portal vein finally drains into the sinusoids, which is the capillary system of the liver. Here, in the sinusoids, blood from the portal vein joins with blood flow from end-arterial branches of the hepatic artery. Once passed through the sinusoids, blood enters the collecting branch of the central vein, and finally leaves the liver via the hepatic vein. The hexagonal structure with, in most cases, three portal canals in its corners draining into one central vein, is defined as a lobule.

The lobule largely consists of hepatocytes (liver cells) which are arranged as interconnected plates, usually one or two hepatocytes thick. The space between the plates forms the sinusoid. A more functional unit of the liver forms the acinus. In the acinus, the portal canal forms the center and the central veins the corners. The functional acinus can be divided into three zones:

1) The periportal zone, which is the circular zone directly around the portal canal,
2) The central zone, the circular area around the central vein,
3) A midzonal area, which is the zone between the periportal and pericentral zone

Liver Function

(1) Vascular	Storing blood,
	Regulating blood clotting,
	Cleansing the blood,
	Discharging west product into bile,
	Aiding immune function
(2) Secretary	Aiding digestion by synthesizing and secreting biles,
	Keeping hormones in balance.
(3) Metabolic	Manufacturing new proteins,
	Regulating fat storage,

Storing Vitamins, Minerals, and Sugars,
Detoxification of Xenobiotic substance e.g. drugs, chemicals etc,
Metabolizing alcohol,
Metabolizing carbohydrates, fats, proteins.

Liver detoxification pathway

The human body identifies almost all drugs as foreign substances (i.e. xenobiotics) and subjects them to various chemical processes (i.e. metabolism) to make them suitable for elimination. This involves chemical transformations to (a) reduce fat solubility and (b) to change biological activity. Although almost all tissue in the body have some ability to metabolize chemicals, smooth endoplasmic reticulum in liver is the principal "metabolic clearing house" for both endogenous chemicals (e.g., cholesterol, steroid hormones, fatty acids, and proteins), and exogenous substances (e.g. drugs). The central role played by liver in the clearance and transformation of chemicals also makes it susceptible to *drug induced injury*. A group of enzymes located in the endoplasmic reticulum, known as cytochrome P-450, is the most important family of metabolizing enzymes in the liver. Cytochrome P-450 is the terminal oxidase component of an electron transport chain. It is not a single enzyme, rather consists of a family of closely related 50 isoforms, six of them metabolize 90% of drugs (1, 2). There is a tremendous diversity of individual P-450 gene products and this heterogeneity allows the liver to perform oxidation on a vast array of chemicals (including almost all drugs) in phase. This important characteristics of the P450 system have roles in drug induced toxicity.

LIVER FUNCTION TEST (LFT)

Liver has to perform different kinds of biochemical, synthetic and excretory functions, so no single biochemical test can detect the global functions of liver. All laboratories usually employ a battery of tests for initial detection and management of liver diseases and these tests are frequently termed “Liver function tests”, although they are of little value in assessing the liver function *per se*. In spite of receiving a lot of criticism for this terminology, the phrase Liver function tests’ is firmly entrenched in the medical lexicon. It might be argued that ‘Liver injury tests’ would be a more appropriate terminology. Moreover, the clinical history and physical

examination play important role to interpret the functions. The role of specific disease markers, radiological imaging and liver biopsy can not be underestimated (3).

IMPORTANCE OF LIVER FUNCTION TESTS

Screening:

They are a non-invasive yet sensitive screening modality for liver dysfunction.

Pattern of disease:

They are helpful to recognize the pattern of liver disease. Like being helpful in differentiating between acute viral hepatitis and various cholestatic disorders and chronic liver disease (CLD).

Assess severity:

They are helpful to assess the severity and predict the outcome of certain diseases like primary biliary cirrhosis.

Follow up:

They are helpful in the follow up of certain liver diseases and also helpful in evaluating response to therapy like autoimmune hepatitis.

CLASSIFICATION AND INTERPRETATION OF LIVER FUNCTION TEST

TESTS OF THE LIVER'S CAPACITY TO TRANSPORT ORGANIC ANIONS AND TO METABOLIZE DRUGS

Serum bilirubin

The catabolism of hemoglobin is outlined in the graphic on the left. Red blood cells are continuously undergoing a hemolysis (breaking apart) process. The average life-time of a red blood cell is 120 days. As the red blood cells disintegrate, the hemoglobin is degraded or broken into globin, the protein part, iron (conserved for latter use), and heme (see Fig.3.13). The heme initially breaks apart into biliverdin, a green pigment which is rapidly reduced to bilirubin, an orange-yellow pigment (see bottom graphic). These processes all occur in the reticuloendothelial cells of the liver, spleen, and bone marrow. The bilirubin is then transported to the liver where it

reacts with a solubilizing sugar called glucuronic acid. This more soluble form of bilirubin (conjugated) is excreted into the bile.

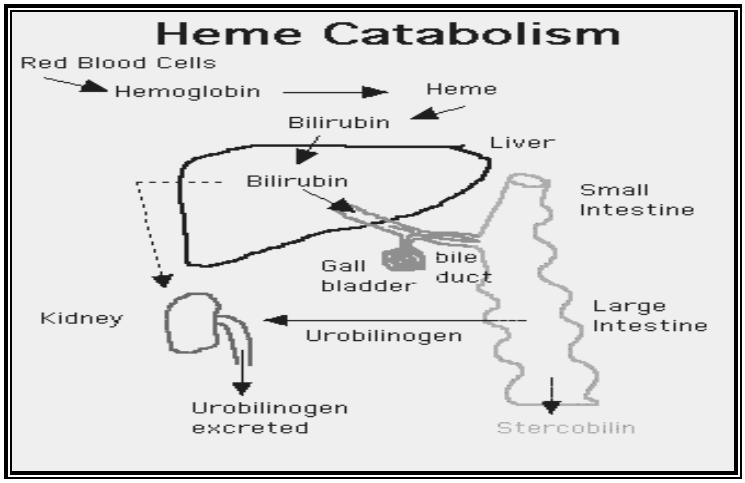


Figure 1. Hemoglobin catabolism and bilirubin

The bile goes through the gall bladder into the intestines where the bilirubin is changed into a variety of pigments. The most important ones are stercobilin, which is excreted in the feces, and Urobilinogen, which is reabsorbed back into the blood. The blood transports the urobilinogen back to the liver where it is either re-excreted into the bile or into the blood for transport to the kidneys. Urobilinogen is finally excreted as a normal component of the urine. The classifications of bilirubin into direct and indirect bilirubin are based on the original van der Bergh method of measuring bilirubin. When the liver function tests are abnormal and the serum bilirubin levels more than 17µmol/L suggest underlying liver disease (4).

Types of bilirubin

i. Total bilirubin: Normal range is 0.2-0.9 mg/dl (2-15µmol/L). It is slightly higher By 3-4 µmol/L in males as compared to females. It is this factor, which helps to diagnose Gilbert syndrome in males easily.

ii. Direct Bilirubin: Normal range 0.3mg/dl (5.1µmol/L)

iii. Indirect bilirubin: This fraction is calculated by the difference of the total and direct bilirubin and is a measure of unconjugated fraction of bilirubin (3, 4).

Diagnostic value of bilirubin levels:

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. Other causes of extreme hyperbilirubinemia include severe parenchymal disease, septicemia and renal failure (5).

Increased unconjugated bilirubin:

This results from overproduction/impaired uptake, conjugation

Increased conjugated bilirubin:

Impaired intrahepatic excretion/ regurgitation of unconjugated or conjugated bilirubin from hepatocytes of bile ducts (6). 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.

TESTS THAT DETECT INJURY TO HEPATOCYTES (SERUM ENZYME TESTS)

1) ENZYMES THAT DETECT HEPATOCELLULAR NECROSIS

Aminotransferases

The aminotransferases (formerly transaminases) are the most frequently utilized and specific indicators of hepatocellular necrosis. These enzymes- aspartate aminotransferase (AST, formerly serum glutamate oxaloacetic transaminase-SGOT) and alanine amino transferase (ALT, formerly serum glutamic pyruvate transaminase-SGPT) catalyze the transfer of the á amino acids of aspartate and alanine respectively to the á keto group of ketoglutaric acid. ALT is primarily

localized to the liver but the AST is present in a wide variety of tissues like the heart, skeletal muscle, kidney, brain nd liver. Whereas the AST is present in both the mitochondria and cytosol of hepatocytes, ALT is localized to the cytosol (7).

The cytosolic and mitochondrial forms of AST are true isoenzymes and immunologically distinct (8). About 80% of AST activity in human liver is contributed by the mitochondrial isoenzyme, Mitochondrial AST is increased in chronic liver disease (9). Their activity in serum at any moment reflects the relative rate at which they enter and leave circulation.

Aminotransferase elevation

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 (2).

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.

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, 2).

AST: ALT ratio

The ratio of AST to ALT is of use in Wilson disease, CLD and alcoholic liver disease and a ratio of more than 2 is usually observed. The lack of ALT rise is probably due to pyridoxine

deficiency. 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.

Mitochondrial AST: Total AST ratio:

This ratio is characteristically elevated in alcoholic liver disease. Abstinence from alcohol improves this ratio. It is also seen to be high in Wilson's disease.

Falsely low aminotransferase levels:

They have been seen in patients on long term hemodialysis probably secondary to either dialysate or pyridoxine deficiency.

Low levels have also been seen in uremia (10, 11).

Other enzymes tests of hepatocellular necrosis

None of these tests have proved to be useful in practice than the aminotransferases.These include glutamate dehydrogenase, isocitrate dehydrogenase, lactate dehydrogenase and sorbitol dehydrogenase.

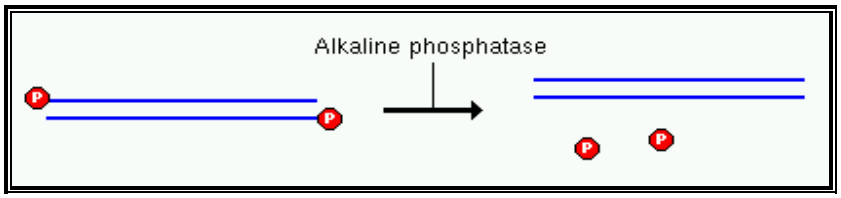
2) ENZYMES THAT DETECT CHOLESTASIS

ALKALINE PHOSPHATASES (ALP)

Alkaline phosphatases 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. In liver, alkaline phosphatase is found histochemically in the microvilli of bile canaliculi and on the sinusoidal surface of hepatocytes. In liver two distinct forms of alkaline phosphatase are also found but their precise roles are unknown. Males usually have higher values as compared to females.

If ALP is high, more tests may be done to find the cause

Alkaline phosphatase removes 5' phosphate groups from DNA and RNA. It will also remove phosphates from nucleotides and proteins. These enzymes are most active at alkaline pH - hence the name.



Highest levels of alkaline phosphatase 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.

The mechanism by which alkaline phosphatase reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions (12) and the other hypothesis is that the damaged liver fails to excrete alkaline phosphatase made in bone, intestine and liver. In acute viral hepatitis, alkaline phosphatase is usually either normal or moderately increased. Hepatitis A may present a cholestatic picture with marked and prolonged itching and elevation of alkaline phosphatase. Tumours may secrete alkaline phosphatase into plasma and there are tumour specific isoenzymes such as Regan, Nagao and Kasahara isoenzymes. Hepatic and bony metastasis can also cause elevated levels of alkaline phosphatase. Other diseases like infiltrative liver diseases, abscesses, granulomatous liver disease and amyloidosis may also cause a rise in alkaline phosphatase. Mildly elevated levels of alkaline phosphatase may be seen in cirrhosis and hepatitis of congestive cardiac failure. Low levels of alkaline phosphatase occur in hypothyroidism, pernicious anemia, zinc deficiency and congenital hypophosphatasia (13). Drugs like cimetidine, frusemide, phenobarbitone and phenytoin may increase levels of alkaline phosphatase.

γ GLUTAMYL TRANSPEPTIDASE (γ GGT)

γGlutamyl transpeptidase (γGGT) is a membrane bound glycoprotein which catalyses the transfer of γglutamyl group to other peptides, amino acids and water. Large amounts are found in the kidneys, pancreas, liver, intestine and prostate. The levels of γGTP are high in neonates and infants up to 1 yr and also increase after 60 yr of life. Men have higher values. Children more than 4 yr old have serum values of normal adults. The normal range is 0-30IU/L (1,3).

In acute viral hepatitis the levels of γglutamyl transpeptidase may reach its peak in the second or third wk of illness and in some patients they remain elevated for 6 weeks. When elevated alkaline phosphatase levels and is unable to differentiate between liver diseases and bony disorders and in such situations measurement of γglutamyl transferase helps as it is raised only in cholestatic disorders and not in bone diseases. 5 In liver disease γglutamyl transpeptidase activity correlates well with alkaline phosphatase levels but rarely the γglutamyl transpeptidase levels may be normal in intra hepatic cholestasis like in some familial intrahepatic cholestasis (14). Other conditions causing elevated levels of γglutamyl transpeptidase include uncomplicated diabetes mellitus, acute pancreatitis and myocardial infarction. Drugs like phenobarbitone, phenytoin, paracetamol, tricyclic antidepressants may increase the levels of γglutamyl transpeptidase. As a diagnostic test the primary usefulness of γglutamyl transpeptidase is limited to the exclusion of bone disease, as γglutamyl transpeptidase is not found in bone.

Some Other Enzyme

Certain tissue cells contain characteristic enzymes which enter the blood only when the cells to which they are confined are damaged or destroyed. The presence in the blood of significant quantities of these specific enzymes indicates the probable site of tissue damage. Figure 3.14 gives a listing of a few enzymes of diagnostic importance and their relationship to the overall metabolic scheme.

1) Creatine Phosphokinase (CPK or CK)

CPK catalyzes the reversible transfer of phosphate groups between creatine and phosphocreatine as well as between ATP and ADP. Most of the CPK resides in skeletal muscle, heart muscle, and in the gastrointestinal tract. CPK enters the blood rapidly following damage to muscle cells. At first CPK seemed to be an excellent marker for acute myocardial infarction (heart damage) or skeletal muscle damage. Unfortunately, the CPK levels rise and fall rapidly and coincide with a variety of other circumstances including surgical procedures, vigorous exercise, a fall, or a deep intramuscular injection. The measurement of CPK levels still provides valuable differentiating diagnostic information.

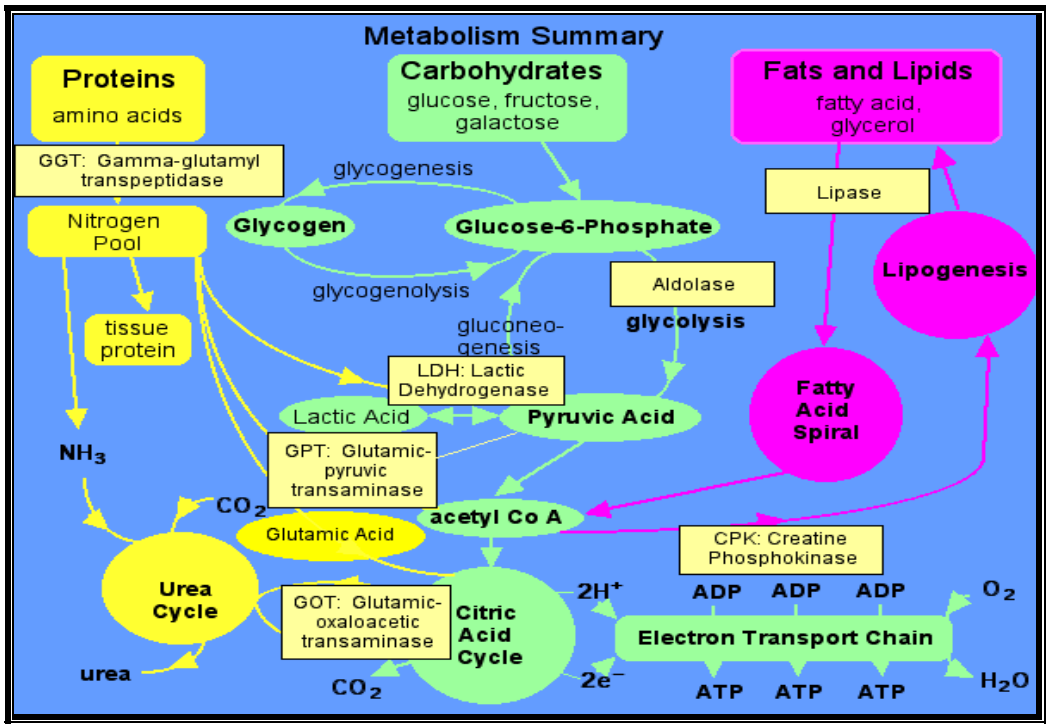


Figure 2 Metabolism summary

2) Lactic Dehydrogenase (LDH):

This enzyme catalyzes the reversible reaction between pyruvic and lactic acids. LDH is present in nearly all types of metabolizing cells, but different cells have different forms of the enzyme which can be distinguished. The enzyme is especially concentrated in the heart, liver, red blood cells, kidneys, muscles, brain, and lungs. The total LDH can be further separated into five components or fractions labeled by number: LDH-1, LDH-2, LDH-3, LDH-4, and LDH-5. Each of these fractions, called isoenzymes, is used mainly by a different set of cells or tissues in the body. The LDH isoenzymes test assists in differentiating heart attack, anemia, lung injury, or liver disease from other conditions that may cause the same symptoms. LDH-1 is found mainly in the heart. LDH-2 is primarily associated with the system in the body that defends against infection. LDH-3 is found in the lungs and other tissues, LDH-4 in the kidney, placenta, and pancreas, and LDH-5 in liver and skeletal muscle. Normally, levels of LDH-2 are higher than those of the other isoenzymes.

Certain diseases have classic patterns of elevated LDH isoenzyme levels. For example, an LDH-1 level higher than that of LDH-2 is indicative of a heart attack or injury; elevations of

LDH-2 and LDH-3 indicate lung injury or disease; elevations of LDH-4 and LDH-5 indicate liver or muscle disease or both. A rise of all LDH isoenzymes at the same time is diagnostic of injury to multiple organs. One of the most important diagnostic uses for the LDH isoenzymes test is in the differential diagnosis of myocardial infarction or heart attack. The total LDH level rises within 24-48 hours after a heart attack, peaks in two to three days, and returns to normal in approximately five to ten days. This pattern is a useful tool for a delayed diagnosis of heart attack. The LDH-1 isoenzyme level, however, is more sensitive and specific than the total LDH. Normally, the level of LDH-2 is higher than the level of LDH-1. An LDH-1 level higher than that of LDH-2, a phenomenon known as "flipped LDH," is strongly indicative of a heart attack.

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