Lecture-3

LIVER FUNCTION TESTS (LFTs)

Liver is a versatile organ which is the central organ of body metabolism and independently involves in many other biochemical functions. Liver perform several diversified functions enumerated below:

1. **Metabolic function:** Liver is the key organ and the principal site where the metabolism of carbohydrate, lipids, protein, minerals and vitamins take place.
2. **Secretory function:** Liver is responsible for the formation and secretion of bile in the intestine. Bile pigment bilirubin formed from heme catabolism is conjugated in liver cells and secreted in the bile. Cholesterol and bile salts are also secreted in the bile into the intestine.
3. **Excretory function:** Exogenous dye BSP (bromosulphthalein) and Rose Bengal dye are excreted through liver cells.
4. **Detoxification and Protective function:** Ammonia is detoxified to urea. Liver cells can detoxified drugs, hormones and convert them into less toxic substances for excretion. Kuffer cells of liver perform phagocytosis to eliminate foreign compounds.
5. **Storage function:** Liver stored glycogen, trace mineral iron and vitamin A, D and B_{12}.
6. **Hematological function:** Liver participates in the formation of blood particularly in the embryo (adults in some abnormal states), synthesis of plasma proteins and blood clotting factors and destruction of erythrocytes.

LFTs : Standard liver function tests

Numerous laboratory investigations have been proposed in the assessment of liver diseases. **"Standard Liver Function Tests (LFTs)"** will help to detect the abnormilities and extent of liver damage.

Classification of LFTs: Classification is based on the specific functions of the liver involved.

1. Tests based on metabolism:

i. Carbohydrate metabolism:
   - Galactose tolerance test
   - Fructose tolerance test

ii. Lipid metabolism:
   - Serum cholesterol: Free and esterified form of cholesterol estimation and their ratio
   - Estimation of fecal fats

iii. Protein metabolism:

- Estimation of total protein, Albumin, Globulin and A/G ratio
- Determination of prothrombin time
- Flocculation tests: Thymol turbidity test, Zinc sulfate test, Colloidal gold test, Cephalin cholesterol flocculation test, Formal gap test.
- Amino acids in urine

2. Tests based on detoxification and protective functions of liver:

i. Conversion of ammonia to urea:

- Estimation of blood urea
- Estimation of blood ammonia

ii. Formation of bilirubin diglucuronate:

- Estimation of serum bilirubin (Direct and Indirect)
- Icteric index
- VD Bergh reaction
- Urinary estimation of bilirubin and uribilinogen

iii. Hippuric test

3. Tests based on excretory functions:

- Bromosulfthalein (BSP) test
- I^{131} Rose Bengal test

4. Tests based on storage functions of liver:

i. Glycogen estimation

ii. Lipid estimation

iii. Estimation of vitamin A, D, B_{12}

iv. Estimation of serum iron and serum iron binding capacity

5. Tests based on serum enzymes derived from liver: Determination of

- Transaminases
- Alkaline phosphatases
- S- nucleotidase
- ϒ-glutamyl trans peptidase

6. Cellular structural studies:

Liver biopsy

The importance of liver function tests are:

1. To assess the severity of liver damage
2. To differentiate different types of jaundice
3. To find out the presence of latent liver diseases.
    - The first group of tests includes regarding secretory, excretory and enzymatic functions are: Serum bilirubin test, bilirubin and urobilinogen in urine, BSP excretion test, Serum alkaline phophatase estimation and SGPT.
    - Second group of tests meant for assessing the protein synthetic functions are: Total protein estimation, A/G ratio and prothrombin time.
    - The final group include that are meant for lipid metabolic functions are: Estimation of serum cholesterol and determination of free and esterified cholesterol ratio.

Tests based on abnormalities of bile pigment metabolism:

1. VD Bergh reaction and Serum Bilirubin: Bilirubin is estimated by VD Bergh reaction involving Diazo reagent. Van Den Bergh reaction consists of two parts, the direct and indirect reactions.

A. Direct reacton: In immediate development of violet colour in 10-30 seconds and delayed direct reaction in which colour appears from 5-30 minutes and then develops slowly to a maximum.

B. Indirect reaction: Indirect reaction is a essentially method for the quantitative estimation of serum bilirubin. Serum diluted with distilled water and methanol added in an amount insufficient to precipitate the proteins, yet sufficient to permit all the bilirubin to react with the diazo-reagent.

Bilirubin as such is insoluble in water while the conjugated bilirubin is soluble and gives direct positive Van den Bergh reaction. Unconjugated bilirubin gives indirect positive Van den Bergh reaction. If the serum contains both unconjugated and conjugated bilirubin in high concentration, the purple colour is produced immediately (direct positive) which is further intensify by the addition of alcohol (indirect positive). This type of reaction is known as “biphasic”.

Interpretation: Normal serum bilirubin level is 0.2-0.6 mg/dl.

Response of VD Bergh reaction can differentiate the jaundice as follow:-

- **In hemolytic/pre-hepatic jaundice** un-conjugated bilirubin increased Hense indirect positive reaction obtained. Occasionally it may be a delayed direct reaction.

- **In obstructive/post-hepatic jaundice** conjugated bilirubin is increased hence an immediate direct positive reaction obtained.
- **In hepatic jaundice** either or both may be present (biphasic reaction).

2. **Bilirubin in urine:** The conjugated bilirubin, is excreted in urine due to water soluble. This is in contrast to unconjugated bilirubin, which is not excreted. Bilirubin in urine can be detected by Gemlin's or Fouchet's test.

Tests based excretory function of liver:

- **Bromosulphthalein test (BSP):** A measured amount of dye is injected intravenously. The liver removes the dye rapidly and excretes in the bile. If the liver function is impaired, the excretion is delayed and larger proportion of dye remains in the serum. It is very sensitive test and is most useful in liver cell damage without jaundice, in cirrhosis and chronic hepatitis. In healthy adults not more than 5% dye should be remain in blood but the bulk of dye is removed in 25 minutes.

  In hepatic diseases, cirrhosis, 40-50 % of dye retension takes place. Also abnormal retention of dye in hepato-cellular or obstructive jaundice takes place.

Tests based metabolic capacity of liver:

- **Galactose tolerance test:** LFT can be assessed by measuring the utilization of galactose. This is known as galactose tolerance test. The animal is given intravenously galactose (about 300 mg/kg body weight). Blood is drawn at 10 minutes intervals for the next 2 hrs and galactose estimated. If the normal individuals half life of galactose is about 10-15 minutes. This is markedly elevated in hpatocellular damage (infective hepatitis, cirrhosis).
- **Serum protein, Albumin and A/G ratio:** Serum protein estimation yields most useful information in chronic liver disease. The liver is the site of albumin, fibrinogen and some of α and β-globulin synthesis. The half life of albumin is about 20-25 days. In advanced liver diseases, the albumin is decreased and globulin is increased so that albumin-globulin ratio is reversed in liver cirrhosis. Serum proteins are decreased in malnutrition and liver damage. Low serum albumin is found in severe liver damage due to impairment ability of the liver to form albumin.
- **Estimation of total and esterified cholesterol:** Liver synthesizes esterified cholesterol and excretes into bile. So cholesterol level is the marker for liver disease. Cholesterol level is decreased in hepatitis, cirrhosis, hyper-thyroidism, malabsorption syndrome in severe wasting in acute infections pernicious anemia etc. Cholesterol level is increased in

obstructive jaundice, intrahepatic obstruction, myxedema, lipid storage disease, atherosclerosis, nephritic syndrome, diabetes mellitus etc.

- **Prothrombin time:** The time required for clotting to take place in citrated plasma to which optimum amounts of thromboplastin and calcium has been added. Prothrombin is formed by the liver cells, vitamin K. When bile salts are not present in the intestine, the absorption of vitamin K form the intestine is impaired. In jaundice and liver disease the prothrombin time is prolonged.

Normal values for icteric index and prothtombin time:

Species	Icteric index	Prothrombin time
Cattle	8.4-9.8	7 minutes
Buffalo	7.1-9.8	9 minutes
Horse	15.5-18.0	11 minutes
Sheep	6.8-10.7	2-3 minutes
Dog	5.8-6.0	4 minutes

Tests based detoxification:

- **Hippuric acid synthesis:** Liver is the major site for detoxification. This test is ideal test for assessing the detoxification function of liver. Hippuric acid is produced in the liver when benzoic acid combines with glycin. 3 gm of hippuric acid, expressed as Benzoic acid or 3.5 gm of sodium benzoate should be excreted in healthy condition. Smaller amounts are found when there is either acute or chronic liver damage. Amount lower than 1 gm may be excreted by patients with infectious hepatitis.

Value of serum enzymes in liver diseases:

A large number of enzyme estimations are available which are used to ascertain liver function.

Most routinely used laboratory tests are two: Serum transaminase (Amino transferase) and Serum alkaline phosphatase.

A. Serum Alkaline Phosphatase: Normal level is 3-13 KA(King-Armstrong) Units/100 ml (23-92 IU/L).

Increased level of alkaline phosphatase is found in post necrotic disease, cirrhosis, carcinoma of liver, obstructive jaundice, hepatocellular jaundice

Liver Biopsy: Histopathological studies of liver biopsy reveal various pathological states of liver cells. Indications for Liver biopsy are fibrosis or neoplasms of liver, in metabolic diseases, arsenic poisoning selenium poisoning and for estimation of Vitamin A, Vitamin E, Cu, Zn, Liver glycogen. **Biopsy is contraindicated in liver abscess.**