Notes

17

LIVER FUNCTION TESTS

17.1 INTRODUCTION

Liver function tests are a group of tests done to assess the functional capacity of the liver as well as any cellular damage to the liver cells. To assess all functional capabilities of the liver such as:

(a) Its Synthetic ability: By measuring the various plasma proteins such as albumin and prothrombin that are synthesized by the liver. Also lipids which are also synthesized in the liver.

(b) Its secretory/excretory abilities: By measuring the serum billirubin level

OBJECTIVES

After reading this lesson, you will be able to:

- enumerate the various tests undertaken to measure liver function and damage.
- describe the various tests are read to reach a conclusion as to the type of damage inflicted on the liver.

17.2 THE COMMON TESTS THAT FORM PART OF THE LIVER FUNCTION TEST PROFILE

(a) Serum Bilirubin: both conjugated and unconjugated.

(b) Total serum proteins and albumin globulin ratio.

(c) Liver enzymes : Transaminases :AST (SGOT), ALT (SGPT). Others: ALP, GGT, LDH.

(d) Prothrombin time

17.3 SERUM BILIRUBIN

Bilirubin is one of the end products of haem metabolism and is derived from the haem part of the hemoglobin molecule. It is a yellow coloured pigment.

Liver plays an important role in the metabolism of bilirubin. After the breakdown of haem portion of the hemoglobin molecule 'unconjugated bilirubin' is insoluble in water. It is transferred from the site of RBC and haem breakdown such as the spleen to the liver for 'conjugation' bound to albumin. At the liver it is conjugated with glucoronic acid with the help of enzyme glucuronyl transferase. This conjugation makes bilirubin water soluble and this conjugated bilirubin is excreted into the bile.

While measuring bilirubin we measure total and conjugated bilirubin (Direct bilirubin) and calculate the indirect bilirubin by substracting the direct from the total.

The normal range of bilirubin is:

Total Bilirubin: 0.2 to 1 mg/dl

Unconjugated Bilirubin: 0.1 to 0.6 mg/dl

Conjugated bilirubin: 0.1 to 0.4 mg/dl

A rise of bilirubin level to that of 2 mg/dl results in the symptoms of jaundice which is marked by deposition of bilirubin in the various mucous membranes.

Jaundice is divided into three types depending on its etiology:

(a) Pre hepatic jaundice: In this case the cause of jaundice is at the level of bilirubin processing before it reaches the liver. Most common cause is over production of bilirubin due to hemolytic disorders. In this case the rise in the level of unconjugated bilirubin is more than conjugated bilirubin hence there is a rise in total and indirect bilirubin.

(b) Hepatic jaundice: This is caused by cellular dysfunction of the liver hence is also called hepatocellular jaundice. It is caused by the inability of the liver cells to process and excrete the bilirubin in the system. It is seen in hepatitis, cirrhosis of liver etc. In this jaundice there is rise in total, direct as well as indirect bilirubin levels.

(c) Post hepatic jaundice: This is also known as obstructive jaundice as it is caused by obstruction to the outflow of bile resulting is reabsoption of conjugated bilirubin and it making an appearance in the serum. It cause by carcinoma of the mouth of gall bladder, stone in the bile duct etc. In this type of jaundice we see a rise in total as well as direct (conjugated) bilirubin.

The common method of measuring serum bilirubin level is the Diazo method using Diazotized sufanilic acid to convert bilirubin into a azobilirubin the color intensity of which is measured colorimetrically at a wavelength between 555 nm (550 to 580 nm).

The conjugated bilirubin reacts directly with the Diazo sulfanilic acid in an aqueous medium and hence also called direct bilirubin. For unconjugated or free bilirubin which is not water soluble we need to dissolve it into DMSO for the reaction to occur. This method is the 'indirect method' for measuring bilirubin levels and it measures both conjugated and unconjugated bilirubin i.e. total serum bilirubin.

INTEXT QUESTIONS 17.1

1. Liver function test assesses ..... of liver
2. The synthetic ability of liver is assessed by measuring .....
3. The secretory ability is assessed by measuring .....
4. Indirect bilirubin is calculated by substracting ..... from .....
5. Serum bilirubin is commonly measured by ..... method

17.4 SERUM ALBUMIN AND ALBUMIN GLOBULIN RATIO

Serum albumin is an important serum protein vital for maintaining the plasma oncotic pressure as well as acts as a carrier for various biological substances and drugs. Serum albumin is exclusively synthesized by liver and hence the level of serum albumin gives us a stock of the synthetic ability of the liver.

Another cause of fall of serum albumin maybe protein malnutrition but in that case the fall of all serum proteins including globulins will be seen.

The normal total protein level is 5 to 8.5 gm/dl. The total serum albumin level is 3.5 to 5 gm/dl. The total plasma globulin level is calculated by subtracting the plasma albumin from the total protein level and is normally in the range of 2 to 2.5 gm/dl.

The normal range for albumin : globulin ratio is 1.2 to 1.5. But with hepatic dysfunction this ratio recedes towards 1 as the synthetic function of liver is compromised. The reversal of the ratio i.e if the value recedes below 1, it is an ominous sign and may mark an infective/inflammatory pathology marked by rise in serum globulin level and fall in serum albumin levels.

To measure serum albumin the Bromocresol green method is used. Albumin in the presence of bromocresol green at a slightly acidic pH gives a yellow green to blue geen colour. The intensity of this colour is dependent on the concentration of albumin in the sample. This intensity is read at a wavelength of 630 nm.

To measure the total protein content of the sample the biuret method it used. In this method the cupric ions of copper (II) sulphate, present in the biuret reagent, form a violet coloured complex with the proteins in a slightly alkaline medium. The intensity of the colour formed is measured at a wavelength of 540 nm (530 to 550 nm).

INTEXT QUESTIONS 17.2

Match the following

1. Total Bilirubin	(a) 5 - 8.5 mg.dl
2. Conjugated Bilirubin	(b) Serum albumin
3. Total Protein	(c) 0.2 - 1 mg/dl
4. Total serum albumin	(d) Total protein
5. Bromocresol green method	(e) 0.1 - 0.4 mg/dl
6. Biuret method	(f) 3.5 - 5 gm/dl

17.5 PROTHROMBIN TIME

Prothrombin is a clotting factor (clotting factor II) and it forms an important part of both the intrinsic and extrinsic pathway. Its active form is Thrombin (also clotting factor IIa). It is a serine peptidase which converts fibrinogen to fibrin. Prothrombin is synthesized in the liver. And hence prothrombin activity in plasma is used to measure the synthetic function of liver.

Prothrombin time is measured by taking human plasma from blood that has been collected in tube containing citrate as an anticoagulant. The plasma in put in an automated machine which adds an excess of calcium to reverse the anticoagulant effects of citrates and measures the time taken for fibrinogen to be converted to fibrin hence measures the activity of thrombin in the plasma.

The prothrombin time differs in accordance to the analytical method used. Hence to compensate for this International normalized ratio (INR) is used. In this the manufacturers of the kit assign an International sensitivity index (ISI) value. This shows the amount of tissue factor present in the kit as against an internationally accepted standard. The ISI value is generally 1 to 2.

The following is the method to calculate INR:

$$INR = \left(\frac{\text{PT test sample}}{\text{PT control}}\right)^{ISI}$$

A ratio of 0.8 to 1.2 is considered normal for patients not on warfarin. For individuals on warfarin for any disorder an INR of 2.0 to 3.0 is the target.

17.6 LIVER ENZYMES

Liver enzymes along with bilirubin are the most commonly measured parameter measured in the liver function test. These enzymes are hepatic in origin and they are leaked into the serum with the destruction of hepatic cells. Liver enzymes are measured to get an idea of the cellular insult on the liver and are increased in a wide variety of conditions such as viral hepatitis, toxic hepatitis, cirrhosis of liver etc.

The commonly measured enzymes are:

(a) Transaminases: AST (SGOT), ALT (SGPT)

(b) Transpeptidases: GGT

(c) Phosphatase: ALP.

(a) Transaminases: They are a group of enzymes that transfer the amino group from an amino acid to α keto acid converting the α keto acid into an amino acid while converting the amino acid into a keto acid.

The transaminases that are measured in the liver function test are ALT and AST.

Alanine transaminase (ALT) catalyses the following reaction:

$$\text{Alanine} + \alpha\text{ keto Glutarate} \xrightarrow{ALT} \text{Pyruvate} + \text{Glutamate}$$

Aspartate transaminase (AST) catalyses the following reaction:

$$\text{Aspartate} + \alpha\text{ keto Glutarate} \xrightarrow{AST} \text{Oxaloacetate} + \text{Glutamate}$$

- The normal level of ALT in serum is 7 to 40 IU/L.
- The normal level of AST in serum is 8 to 40 IU/L.

An increase in AST or ALT levels hints at an insult to the liver parenchyma tissue. ALT is a more specific marker of hepatic injury than AST as AST elevation is also seen in cardiac tissue injury, haemolysis and muscle tissue.

To measure the level of transaminases the reaction catalysed by them is coupled to a reaction in which NADH is used up resulting in change in the photometric intensity when read in the UV range at 340 nm. It is a UV kinetic method.

For ALT (SGPT):

$$\text{Alanine} + \alpha\text{ Keto glutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow[\text{(Lactate dehydrogenase)}]{\text{LDH}} \text{Lactate} + NAD^+$$

For AST (SGOT):

$$\text{Aspartate} + \alpha\text{ Keto glutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow[\text{(Malate dehydrogenase)}]{\text{MDH}} \text{Malate} + NAD^+$$

(b) Alkaline Phosphatase: It is a hydrolase that removes phosphates from all kinds of molecules such as proteins, nucleotides etc.

It is found in cells lining the billiary system hence a rise in it level is indicative of damage to the billiary tree due to cholestasis. It maybe due to stone blocking the large ducts or intrahepatic obstruction, inflammation of the biliary channels.

Alkailine phosphatase is also found in placenta and bones. Hence the level is also increased in growing children in whom bones undergo remodeling and in Paget's disease in adults.

Normal level of alkaline phosphatase is between 45 to 115 IU/L.

The method for measuring the level of alkaline phosphatase is a kinetic method using p-nitrophenylphosphate as substrate for the enzyme and measuring rate of formation of the colored substrate (p- nitrophenol) formed from the reaction. This measurement of the color intensity is done colorimetrically at a wavelength of 405 nm.

$$\text{p-Nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{p- Nitrophenol} + \text{Phosphate}$$

(c) Gamma glutamyl transpeptidase: It is another enzyme specific to the biliary tree and a more specific indicator of cholestasis and damage to the biliary tree. It is also a highly specific marker and is raised in even minute and subclinical damage to the biliary tree.

Its normal range is in between 0 to 42 IU/l.

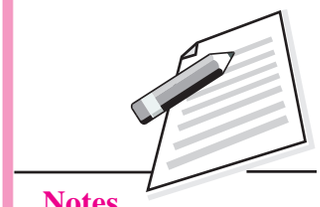
INTEXT QUESTIONS 17.3

Match the following

1.	AST	(a)	45 - 115 IU/L
2.	Alkaline Phosphatase	(b)	0 - 42 IU/L
3.	ALT	(c)	8 - 40 IU/L
4.	Gamma glutamyl transpeptidase	(d)	7 - 40 IU/L

WHAT HAVE YOU LEARNT

- Liver function tests are a group of tests done to assess the functional capacity of the liver
- Synthetic ability of the liver is assessed by measuring the various plasma proteins
- Secretory/excretory ability is assessed by measuring the serum billirubin level
- The common tests that form part of the liver function test profile are Serum Bilirubin both conjugated and unconjugated, Total serum proteins and albumin globulin ratio, Liver enzymes and Prothrombin time
- While measuring bilirubin we measure total and conjugated bilirubin (Direct bilirubin) and calculate the indirect bilirubin by substraction the direct from the total.
- The common method of measuring serum bilirubin level is the Diazo method
- The normal total protein level is 5 to 8.5 gm/dl. The total serum albumin level is 3.5 to 5 gm/dl and the normal range for albumin : globulin ratio is 1.2 to 1.5
- Serum albumin is measured by Bromocresol green method and total protein is measured by biuret method
- Prothrombin is a clotting factor (clotting factor II) and it forms an important part of both the intrinsic and extrinsic pathway
- International normalized ratio (INR) is used for measuring the Prothrombin time
- The commonly measured enzymes are Transaminases: AST (SGOT), ALT (SGPT), Transpeptidases: GGT, Phosphatase: ALP.



TERMINAL QUESTIONS

1. What are functional aspects of liver?
2. Write a short note on conjugated and unconjugated bilirubin.
3. Write short note on the laboratory method to measure serum bilirubin level both direct and indirect.
4. What is the method used to measure albumin in serum?
5. What is the method used to measure total protein in serum?
6. What is the importance of INR and how is it calculated?

ANSWERS TO INTEXT QUESTIONS

17.1

1. Functional capacity
2. Plasma protein
3. Serum bilirubin
4. Direct, total bilirubin
5. Diazo

17.2

1. (c) 2. (e) 3. (a) 4. (f) 5. (b) 6. (d)

17.3

1. (c) 2. (a) 3. (d) 4. (b)