Liver Function Tests

PRESENTED BY:

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LIVER FUNCTION TEST

Liver Function Test is group of blood tests that reveals whether your liver is functioning properly or

not.

Broadly classified as:

1. Tests to detect hepatic injury:

- Mild or severe; acute or chronic
- Nature of liver injury (hepatocellular or cholestasis)
- 2. Tests to assess hepatic function



CLASSIFICATION

Group I: Markers of liver dysfunction

- Serum bilirubin: total and conjugated.
- Total protein, serum albumin and albumin/globulin ratio

Group II: Markers of hepatocellular injury

- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)

Group III: Markers of cholestasis (obstructive)

- Alkaline phosphatase (ALP)
- g-glutamyl transferase (GGT)

PURPOSE OF THE TEST (INDICATIONS)

Liver function tests can be used to:

- Screen for liver infections, such as hepatitis
- Monitor the progression of a disease, such as viral or alcoholic hepatitis, and determine how well a treatment is working
- Measure the severity of a disease, particularly scarring of the liver (cirrhosis)
- Monitor possible side effects of medications
- Liver function tests check the levels of certain enzymes and proteins in your blood. Levels that are higher or lower than normal can indicate liver problems

What tests are included in routine LFT

- ▶ Bilirubin.
 - * Bilirubin total
 - * Bilirubin direct and indirect.
- ► SGPT (ALT)
- ► SGOT(AST)
- ► Alkaline phosphatase(ALT)
- Total protein.
- Albumin.
- ► A/G ratio
- ► Gamma-glutamyltransferase (GGT).

- ▶ **Bilirubin.** Bilirubin is a substance produced during the normal breakdown of red blood cells. Bilirubin passes through the liver and is excreted in stool. Elevated levels of bilirubin might indicate liver damage or disease or certain types of anemia.
- ▶ Alanine transaminase (ALT). ALT is an enzyme found in the liver that helps in conversion of proteins into energy for the liver cells. When the liver is damaged, ALT is released into the bloodstream and levels increase.
- Aspartate transaminase (AST). AST is an enzyme that helps in metabolism of amino acids. Like ALT, AST is normally present in blood at low levels. An increase in AST levels may indicate liver damage, disease or muscle damage.

- ▶ Alkaline phosphatase (ALP). ALP is an enzyme found in the liver and bone and is important for breaking down proteins. Higher-than-normal levels of ALP may indicate liver damage or disease, such as a blocked bile duct, biliary cirrhosis, obstructive jaundice etc , or certain bone diseases(paget disease, osteomalacia etc.)
- ▶ Albumin and total protein. Albumin is one of several proteins made in the liver. Human body needs these proteins to fight infections and to perform other functions. Lower-than-normal levels of albumin and total protein may indicate liver damage or disease(acute hepatitis, chronic liver disease, dehydration, etc)

► Gamma-glutamyltransferase (GGT). GGT is an enzyme in the blood. Higher-than-normal levels may indicate liver or bile duct damage.

▶ L-lactate dehydrogenase (LD). LD is an enzyme found in the liver, cardiac muscles, kidney, skeletal muscles and other tissues. Elevated levels may indicate liver damage but can be elevated in many other disorders(myocardial infraction, pernicious anemia etc)

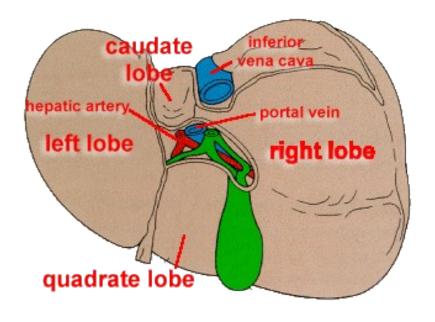
Normal values

Test	Normal Range
Bilirubin Total	0-1 MG/DL
Conjugated (D. Bilirubin)	0-0.35 MG/DL
Unconjugated (I.D. Bilirubin)	0.2-0.65 MG/DL
SGOT	10-40 IU/L
SGPT	10-40 IU/L
Alkaline Phosphatase	40-112 U/L
Total Protein	6-8.5 GM/DL
Albumin	3.5-5 GM/DL
Globulin	2-3.5 GM/DL Healthcheckup.com

PATHOPHYSIOLOGY

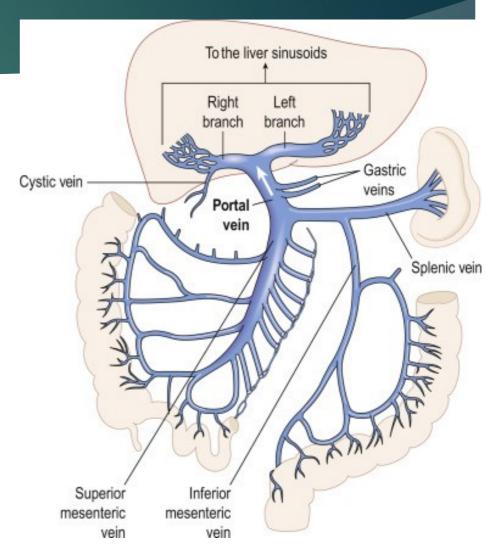
Liver: Introduction:

- A human liver normally weights 1.44–1.66 kg, and is a soft, pinkishbrown, triangular organ.
- ☐ The liver is both the largest internal organ (the skin being the largest organ overall) and the largest gland in the human body.
- ☐ The liver is connected to two large blood vessels: the hepatic artery and the portal vein.
- ☐ Traditionally, the liver is divided into four lobes: left, right, caudate, and quadrate. The lobes are further divided into lobules, the functional units of the liver.
- Each lobule is made up of millions of hepatic cells that are the basic metabolic cells of the liver.



BLOOD SUPPLY TO THE LIVER

- In the hepatic portal system, the liver receives a dual blood supply from the hepatic portal vein(the union of the superior mesenteric and splenic veins) carries venous blood from the GI tract to the liver. and the hepatic arteries carry oxygenated blood to the liver.
- ► The hepatic portal vein supplies 75% of the blood to the liver, while the hepatic arteries supply the remaining 25%.



FUNCTIONS OF LIVER

- It can **store** (**storage function**):
 - Amino Acids.
 - Carbohydrates.
 - Lipids.
 - Vitamins.
 - Minerals.
- It can synthesize (metabolic function):
 - ▶ Protein, like albumin, alpha and beta globulins.
 - ► Coagulation factors.
 - Transport proteins.
 - ▶ Bile acids from cholesterol.

- The liver is the site for **detoxification** of:
 - drugs.
 - Toxins.
- ► Its major function is **Conjugation**:
 - bilirubin combine with glucuronic acid as:
 - Bilirubin Monoglucuronide.
 - ▶ Bilirubin diglucuronide.

- The liver has an **excretory function**:
 - Excrete bilirubin into bile.
 - ▶ Bile acid is excreted into the bile.
- The liver is also the site of **catabolism** of:
 - ► Thyroid hormone.
 - ► Steroids hormones.

BILIRUBIN

- Bilirubin is the water insoluble breakdown product of normal heme catabolism
- It's a yellow pigment present in bile (a fluid made by the liver), urine and feces.
- Heme is found in hemoglobin, which is a principal component of RBCs [Heme: iron + organic compound "porphyrin"].

Source of Heme in body:

- ► 80% from hemoglobin
- 20% other hemo-protein: cytochrome, myoglobin)

TYPES OF BILIRUBIN IN SERUM:

- ✓ Indirect bilirubin: is unconjugated (water insoluble bilirubin). Because of it's less solubility in water, it reacts more slowly with reagent (reaction carried out in methanol). Unconjugated bilirubin will calculated by subtracting direct from total and so called indirect.
- Direct bilirubin: is conjugated (water soluble bilirubin). In aqueous solution it reacts rapidly with reagent (direct reacting).
- ✓ Total bilirubin = D+ ID

DIFFERENCE B/W CONJUGATED AND UNCONJUGATED BILIRUBIN

Conjugated Bilirubin

- Present normally in bile.
- Conjugated to glucoronic acid.
- Can be filtered by kidney.
- Cannot cross blood brain barrier.

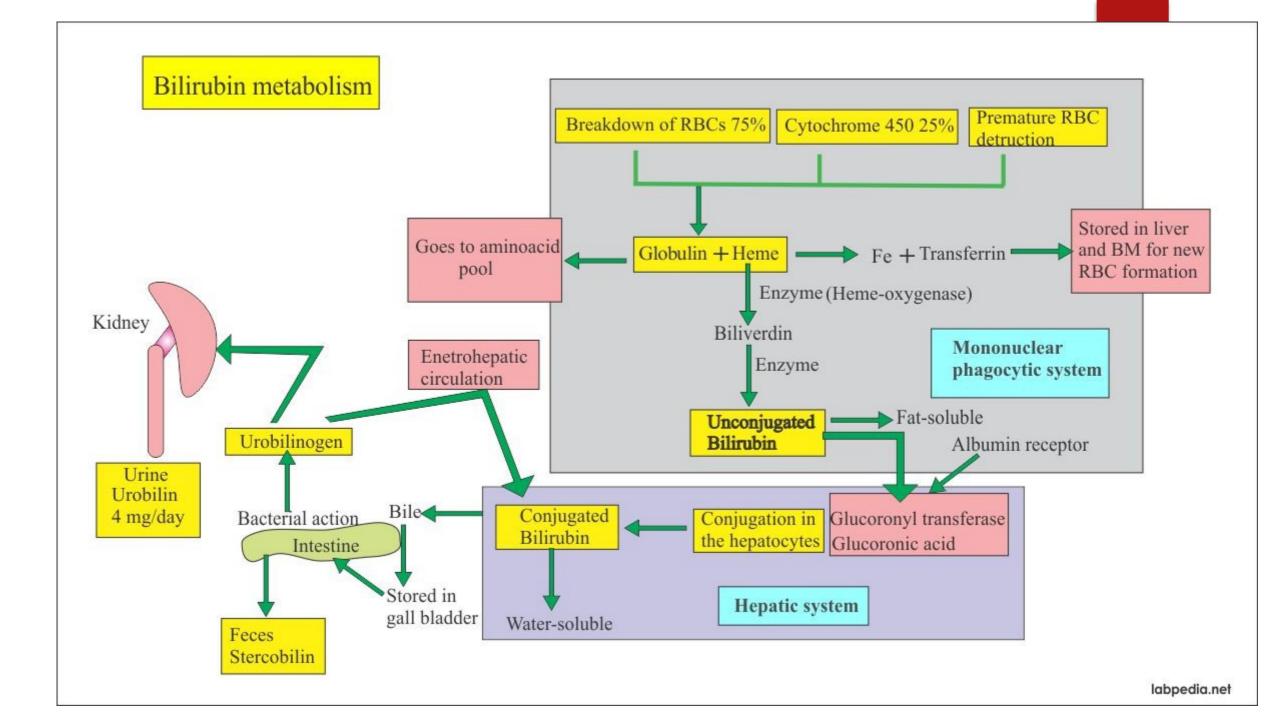
Unconjugated Bilirubin

- Present normally in plasma.
- Conjugated to albumin.
- Cannot be filtered by kidney.
- Can cross BBB.

HOW BILIRUBIN FORMS:

Changes in the mononuclear phagocytic system:

- Breakdown of the RBCs is a major source for the formation of Globin and Heme.
- Heme changes into Biliverdin and releases iron which is recycled for the formation of RBCs in the bone marrow.
- Biliverdin forms the unconjugated bilirubin which is fat soluble.



Raised Bilirubin level is seen in:

- 1. Some infections, such as an infected gallbladder, or cholecystitis.
- 2. Some inherited diseases, such as Gilbert's syndrome.
- 3. Diseases that cause liver damage, such as hepatitis, cirrhosis, or mononucleosis.
- 4. Diseases that cause blockage of the bile ducts, such as gallstones or cancer of the pancreas.
- 5. Rapid destruction of red blood cells in the blood, such as from sickle cell disease or an allergic reaction to blood received during a transfusion (called a transfusion reaction).
- 6. Medicines that may increase bilirubin levels. This includes many antibiotics, some types of birth control pills, indomethacin (Indocin), phenytoin (Dilantin), diazepam (Valium), and flurazepam (Dalmane).

Decreased Bilirubin level is seen in:

1. Medicines that may decrease bilirubin levels. This includes vitamin C (ascorbic acid), phenobarbital, and theophylline.

Causes of Conjugated Hyperbilirubinemia

1.Decreased secretion into bile canaliculi:

- * Hepatitis.
- * Cholestasis (Intrahepatic).
- **Dubin Johnson syndrome.**
- * Rotor syndrome.

2.Decreased drainage or excretion.

- 1. Extra hepatic obstruction:
 - Stones.
 - Carcinoma.
 - Stricture.
 - * Atresia.

- 2. Sclerosing cholangitis.
- 3. Intrahepatic obstruction:
 - Drugs.
 - primary biliary cirrhosis.
 - * Tumors.
 - Granulomas.
 - Idiopathic neonatal hepatitis (cholestatic jaundice).

Causes of Indirect hyperbilirubinemia

1. Increased production:

- * Hemolysis (Hemolytic disease of the new born).
- * Hereditary.
- * Acquired.
- Ineffective erythropoiesis.
- Increased turn over like in neonates.
- Physiologic jaundice of new born.

2.Decreased delivery:

- Congestive heart failure.
- Portacaval shunt.

3.Decreased uptake by the hepatocytes:

- Drugs.
- **Gilbert's syndrome.**
- * Sepsis.
- * Fasting.

4.Decreased conjugation:

- 1. Physiologic jaundice e.g. Neonatal jaundice.
- 2. Drugs.
- 3. Hereditary like Crigler-Najjar syndrome.
 - Complete enzyme deficiency, Type 1
 - Partial enzyme deficiency, Type 2
- 4. Hepatocellular dysfunction.
- 5. Gilbert's syndrome.

Methods to determine Sr. Bilirubin

- ▶ 1. By Ven Den Bergh reaction method.
- ▶ 2. By DMSO(dimethyl sulfoxide) method.
- ▶ 3. By Autoanalyzers.

1. Van den Bergh reaction test

- ▶ Van den Bergh reaction is a chemical reaction used to measure <u>bilirubin</u> levels in blood. More specifically, it determines the amount of conjugated bilirubin in the blood.
- The reaction produces <u>azobilirubin</u>.
- ▶ **Principle:** bilirubin reacts with diazotised <u>sulphanilic</u> acid to produce purple coloured Azo bilirubin.
- ▶ this reaction is highly useful in understanding the nature of jaundice. This test helps to identify the type of jaundice.

- The serum of patient is mixed with diazo reagent. If purple colour develops immediately it is called **direct positive**. It happens if conjugated bilirubin is present.
- In **indirect positive test**, patient's serum is first treated with **alcohol** and later mixed with diazo reagent. This causes development of purple colour. It is seen if unconjugated bilirubin is present.
- If both conjugated and unconjugated bilirubin are present the reaction is termed biphasic reaction

PROCEDURE MEASURING SERUM BILIRUBIN LEVEL

Principle:

Sulphanalic acid + NaNO3

diazotized sulphanalic acid (DSA)

DSA + Bilirubin "D"

Azobilirubin "purple"

Bilirubin "ID"+ DSA + accelerator

Total bil.

□Kit components

- Sulfanalic acid reagent
- Sodium nitrate reagent
- ➤ Methanol reagent
- ➤ Bilirubinequavalent standard (5mg/dl T.bil; 2.5 mg/dl D.bil)

Procedure

	Total test	Total blank	Direct test	Direct blank
Distilled water, ml	1.8	1.8	1.8	1.8
Serum, ml	0.2	0.2	0.2	0.2
Diazo mixture, ml	0.5	_	0.5	_
Diazo blank reagent ml		0.5		0.5
Methanol, ml	2.5	2.5	_	_
Distilled water, ml			2.5	2.5

Keep in dark for 30 minutes. Read the intensities at 540 nm (green filter).

Read O.D. of the artificial bilirubin standard (undiluted) by transferring the standard solution in a dry cuvette at 540 nm (or Green filter).

Calculations

Serum bilirubin, mg/dl (total)

$$= \frac{O.D. TT}{O.D. Std.} \times 10$$

Serum bilirubin, mg/dl (direct)

$$= \frac{O.D. TT}{O.D. Std.} \times 10$$

Serum indirect bilirubin, mg/dl = Total bil- direct bil

Normal range : mg/dl Total bilirubin : 0.2-1.0 Direct bilirubin : Up to 0.5

2. DMSO(dimethyl sulfoxide) methods.

Reagents:

- 1. Diazo A "DMSO": 5.0g sulfanilic acid, 12ml HCL, 512 ml dimethyl sulfoxide in 1 lit. distilled water.
- 2. Sulfanilic acid: 0.5g/dl in 1.5% hcl.
- **3. Diazo B**:0.5g/dl, sodium nitrite in distilled water.
- 4. 10mg/dl Bilirubin artificial standard(methyl red or cobalt nitrate)

Procedure

Pipette in the four tubes labeled as follows:

Table 15.6

	Total test	Total blank	Direct test	Direct blank
Reagent (1), ml	2.8	2.8	-	_
Reagent (2), ml	_	_	2.8	2.8
Reagent (3), ml	0.1	_	0.1	_
Serum, ml	_	0.1	_	0.1

Mix thoroughly, and read intensities of total test & direct test against respective blanks at 555 nm (green filter: 530–580 nm).

Calculations

Total bilirubin, mg/dl

$$= \frac{\text{O.D. total test} - \text{O.D. total blank}}{\text{O.D. of std.}} \times 10^{-10}$$

Direct bilirubin, mg/dl

$$= \frac{\text{O.D. direct test - O.D. direct blank}}{\text{O.D. of std.}} \times 10^{-10}$$

3. By Autoanalyzers

Principle:

- 1. Bilirubin reacts with diazotised sulphanilic acid in acidic medium to form pink colored azobilirubin with absorbance directly proportional to bilirubin concentration.
- 2. Direct bilirubin (water soluble) directly reacts in acidic medium.
- 3. Indirect bilirubin is solubilised using surfactant.

Method: Diazo method.

Requirements: Reagent composition

Reagent 1 : Total Bilirubin reagent

Surfactant -1.00%

HCl - 100 m mol/lit.

Sulphanilic acid -5m mol/lit

Reagent 2: Direct bilirubin reagent

Sulphanilic acid -10 m mol/lit

HCl -100 m mol/lit

Reagent 3: Sodium Nitrite reagent

Sodium nitrite - 144 m mol/lit

Reagent preparation

Test Vol. of		add			
	working reagent	Reagent 1	Reagent 2	Reagent 3	
Total bilirubin	10ml	10 ml	-	0.2 ml	
	25 ml	25 ml	-	0.5 ml	
	50 ml	50 ml	-	1.0 ml	
	100 ml	100ml	-	2.0 ml	
Direct bilirubin	10ml	-	10 ml	0.1 ml	
	25 ml	-	25 ml	0.25 ml	
	50 ml	-	50 ml	0.5 ml	
	100 ml	-	100ml	1.0 ml	

To determine Sr. Alkaline Phosphatase

Principle: 2-amino-2-methyl-1-propanol + P-nitrophenylo phosphate+ H2O ALP(Alkaline Phosphatase)

4-nitrophenol+2-amino-2-methyl-1-propanol phosphate.

The rate of 4-nitrophenol formation is directly proportional to the ALP activity at 405nm.

Method: Kinetic method.

▶ Reagent composition

2-amino-2-methyl-1-propanol (AMP)

- 350 m mol/lit.

 Mg^2+

- 2.0m mol/lit.

 Zn^2+

- 1.0 m mol/lit.

HEDTA

-2.0 m mol/lit.

P-nitrophenylo phosphate

- 16.0 m mol/lit.

Increased level of ALP

Liver disease		Bone disease		Other disease
Marked ↑se	Moderate ↑se	Marked ↑se	Moderate ↑se	
Obstructive jaundice	Hepatitis	Paget disease	Osteomalacia	Hyperparathyroidism
Hepatocellular cirrhrosis	Cirrhrosis of liver	Metastatic bone disease	Rickets	Infectious mononucleosis
Biliary cirrhrosis		Osteitis deformans		

Decreased level of ALP

- Hypo phosphatasia, malnutrition
- Hypothyroidism , Pernicious Anemia.
- Scurvy
- Milk alkali syndrome.
- Placental insufficiency.

Serum Albumin

Principle:

Albumin binds with bromocresol green(BCG) at pH 4.2 Causing a shift in absorbance of the yellow BCG dye. The blue-green color formed in proportional to the concentration of albumin present, when measured photometrically b/w 580-630 nm with max. absorbance at 625 nm.

* **Method**: BCG dye method

* Reagent composition

Bromocresol green 0.08 m mol/lit.

Succinate buffer 50 m mol/lit.

Sodium azide 1 gm/ dl

Surfactant ---

Albumin standard 3.6 gm/ dl

Clinical significance of Sr. Albumin

Hypoalbuminemia	Hyperalbuminemia
Nephrotic syndrome	Dehydration
Burns	High protein diet
Blood loss	False value due to prolonged tourniquet
Malignancies	
Inflammatory process	
Liver diseases	
Decreased protein intake	
Ascites	

Serum ALT/S.G.P.T Level

Principle:

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L-Alanine+2-Oxoglutarate ALT Pyruvate + L-Glutamate

Pyruvate + NADH LDH L-Lactate +NAD

ALT(alanine transaminase)

LDH(lactate dehydrogenase)
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- ▶ **Method**: International Federation of clinical chemistry(IFCC) method.
- ► Reagent : SGPT reagent

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L-Alanine -500 m mlo/lit
NADH -0.18 m mol/lit.
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LDH -1820 IU/lit.

2-Oxoglutarate -12 m mol/lit.

Tris buffer -80 m mol/lit.

Incresed ALT level

- 1. <u>Hepatitis</u>, which is an inflammatory condition of the liver
- 2. <u>Cirrhosis</u>, which is severe scarring of the liver
- 3. Death of liver tissue
- 4. A tumor or <u>cancer in the liver</u>
- 5. A lack of blood flow to the liver
- 6. <u>Hemochromatosis</u>, which is a disorder that causes iron to build up in the body
- 7. Mononucleosis, which is an infection usually caused by the Epstein-Barr virus
- 8. <u>Pancreatitis</u>, which is an inflammation of the pancreas
- 9. Diabetes

Serum S.G.O.T/ AST Level

Principles:

- L-Aspartate + 2 Oxoglutarate → AST → Oxaloacetate + Glutamate Oxaloacetate + NADH + H+L- malate + NAD + H2O
- 1.AST catalyse the transfer of amino group between aspartate and 2 oxoglutarate.
- 2. The formesd oxaloacetate is then react with NADH+ H by malate dehydrogenase (MDH) to form NAD.
- 3.AST activity is determined by rate of oxidation of NADH at 340nm.

Increased SGOT level

- Fulminant type of hepatitis.
- In liver cell necrosis or injury like chronic hepatitis,
- Alcoholic hepatitis.
- Liver metastasis
- Severe exercise.
- Severe burn.
- Hypothyroidism (in 40 to 90% of cases).
- In cholestatic and obstructive jaundice.

- Other diseases
 - Shock.
 - Mushroom poisoning.
 - Pulmonary embolism and infarction.
 - Hemolytic anemia.
 - malignant hyperthermia etc.

Decreased SGOT level seen in:

- 1. Chronic renal dialysis.
- 2. Acute renal diseases leading to Azotemia.
- 3. Vit. B6 deficiency.
- 4.Beriberi.
- 5. Pregnancy.

