LIVER FUNCTION TESTS (LFT)

Suman Kumar Mekap
Asst. Professor in Pharmacology
RIPS, Berhampur

What is Liver function test (LFT)?

- Liver function test is a group of blood tests commonly performed to evaluate the function of the liver

- This test measures the level of

  - Liver enzymes
  - Proteins

and

  - Bilirubin in the blood

Why is Liver Function Test (LFT) done?

Liver function test is done:

- As a part of preventive health checkup
- To diagnose liver diseases like viral hepatitis, alcoholic hepatitis, autoimmune hepatitis or liver cirrhosis
- To monitor the efficacy of a therapy given for the treatment of existing liver disease
- To monitor the health of liver when a patient is on medicines with known harmful effects on liver

FUNCTIONS OF LIVER:

- Liver is the largest Organ of the body weighing about 1.5kg.

- Liver is called kitchen of our body.

- **Carbohydrate Metabolism**

In **fed state glycogen** synthesis and excess glucose is converted to fatty acid and then TAGS which get incorporated to VLDL and transported to adipose tissue.

- In **Fasting state** glucose concentration is maintained by glycogenolysis and gluconeogenesis

- **Protein Metabolism:**
- 1. Synthesis of albumin and various plasma proteins except immunoglobulins.

- Most of the coagulation factors like fibrinogen, Prothrombin(II), V, VII, IX , X , XI, XII, XIII

- Out of these II , VII ,IX, X cannot be synthesized with out vitamin –K.
- Transport proteins – eg: Transferrin

- **Amino Acid Metabolism & Urea Formation**

- **Lipid Metabolism:**

- Synthesis of lipoproteins, Phospholipids, Cholesterol.
- Fatty acid Metabolism – ß-Oxidation , Ketone body formation, Bile acid synthesis.

- **Excretion and Detoxification:**
- Conjugation and Excretion of bilirubin
- Cholesterol is excreted in the bile as bile acids and cholesterol.
- Steroid hormones are metabolized and inactivated by conjugation with glucuronic acid and sulphate and are excreted in Urine.

- Drugs are metabolised and inactivated by CYT P450 of endoplasmic reticulum and excreted through bile / urine.

Miscellaneous function:

- Iron storage, vitamins A D E storage, B12 storage.

- Note: Liver has very large functional reserve.
- Deficiencies of Synthetic functions can only be detected if liver disease is very extensive.

How is Liver Function Test (LFT) done?

- LFT is done on a blood sample
- A syringe attached to a fine needle is used to withdraw the blood from a vein of your arm

LFTs are classified as:

- • **Excretory function tests:**
- Bile pigments, salts, acids, bilirubin and BSP
- • **Metabolic functions tests :**
- Carbohydrates, Protiens, Fats
- • **Synthetic capabilities :**
- Proteins(albumin), coagulation factors
- • **Detoxification :**
- Ammonia, drugs
- • **Tests of liver injury :**
- Enzyme assays, autoimmune markers, markers of hepatitis virus infections

Various components of Liver Function test (LFT)

It includes

- 1.Alkaline Phosphatase (ALP)
- 2.Alanine Transaminase (ALT)
- 3.Aspartate Aminotransferase (AST)
- 4.Gamma Glutamyl Transferase (GGT)
- 5.Protein- Total, Serum Albumin
- 6.Globulin, Serum
- 7.Bilirubin - Total, Direct and Indirect

Alkaline phosphatase (ALP)

- High levels of ALP are seen in patients with
- ○ Bile duct obstruction
- ○ Hepatitis
- ○ Cirrhosis
- ○ Liver cancer
- ○ Bone diseases

- High levels of ALP are considered normal in growing children and pregnant women

Alanine Aminotransferase (ALT)

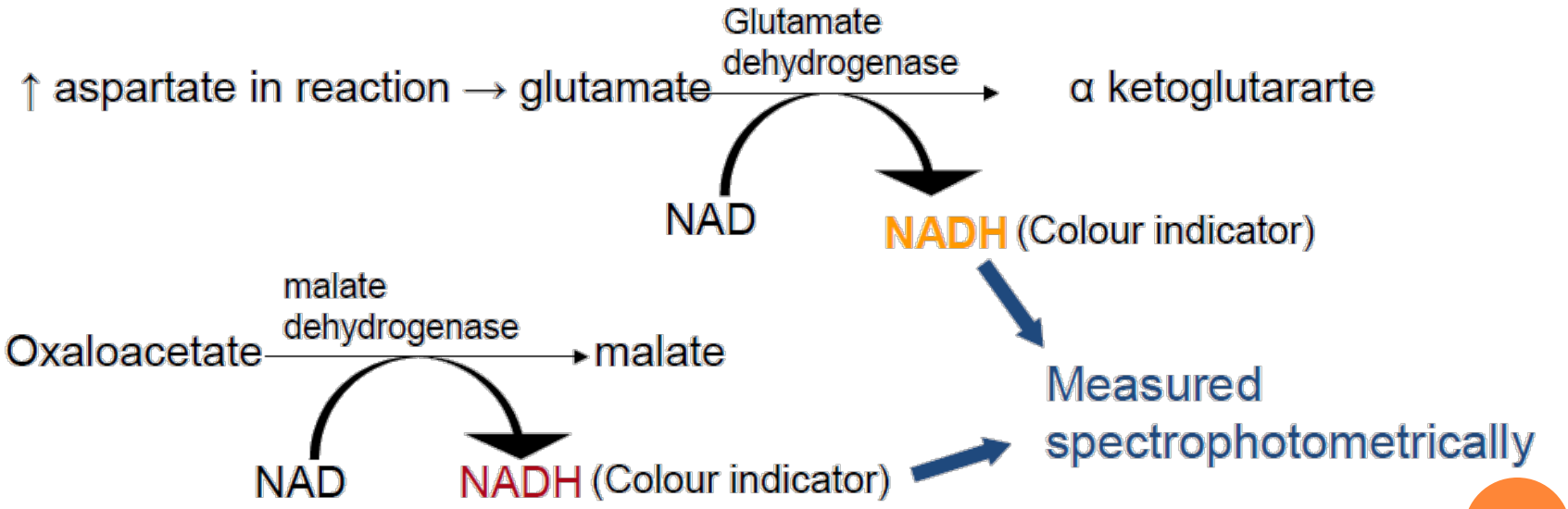
- ● ALT is also known as serum glutamate-pyruvate transaminase (SGPT)
- ● Very high levels of ALT are seen in patients with acute hepatitis
- ● Moderately high levels of ALT are seen in patients with
- ○ Chronic hepatitis
- ○ Blocked bile ducts
- ○ Cirrhosis
- ○ Liver cancer

Aspartate Aminotransferase (AST)

- ● AST is also known as serum glutamic oxaloacetic transaminase (SGOT)
- ● High levels of AST are seen in patients with liver and muscle damage
- ● However AST is not specific for liver damage and is measured along with ALT to diagnose a suspected liver disease

Assay for AST & ALT

- • Vit B6 important requirement for AST and ALT assays
- • Normal serum levels up to 40 IU/dl for both



GAMMA-GLUTAMYL TRANSFERASE (GGT)

- **High levels of GGT are seen in patients with**

- ○ Alcoholic liver disease
- ○ Obstructive liver disease
- ○ Acute and chronic viral hepatitis
- ○ Fatty liver disease
- ○ Cirrhosis
- ○ Cholestasis
- ○ Liver tumors

GGT Assay: substrate - ɣ glutamyl-p nitroanilide → **p nitroaniline** liberated (chromogenic) – measured spectro photometrically

Serum Proteins

- Albumin is the main protein synthesized

- Low levels of albumin indicates that liver is not functioning properly and can be seen in patients with

- ○ Cirrhosis
- ○ Chronic Hepatitis
- ○ Poor nutrition
- ○ Kidney disease
- ○ Infections

GLOBULIN

- Low level of Globulin is seen in malnutrition
- High levels of Globulin are seen in
  - Chronic active hepatitis
  - Alcoholic hepatitis
  - Chronic inflammatory diseases
- Low albumin to globulin ratio
  - Indicates liver cirrhosis
- High albumin to globulin ratio
  - Suggests decreased globulin production
  - Is seen in some genetic diseases or leukemia

Bilirubin

- **High levels of bilirubin are seen in patients with**
- Hepatitis
- Liver cirrhosis
- Bile duct obstruction
- Gallbladder cancer
- Gallstones
- Genetic diseases like Gilbert's syndrome

NORMAL VALUES

- **LFT :**
- Total Bilirubin 0.2 to 0.8 mg/dl
- Conjugated bilirubin 0 to 0.2 mg/dl
- Total protein 6 – 8 gm/dl
- Albumin 3.5 – 5 gm/dl
- Coagulation Factors – PT- 11 to 12 seconds

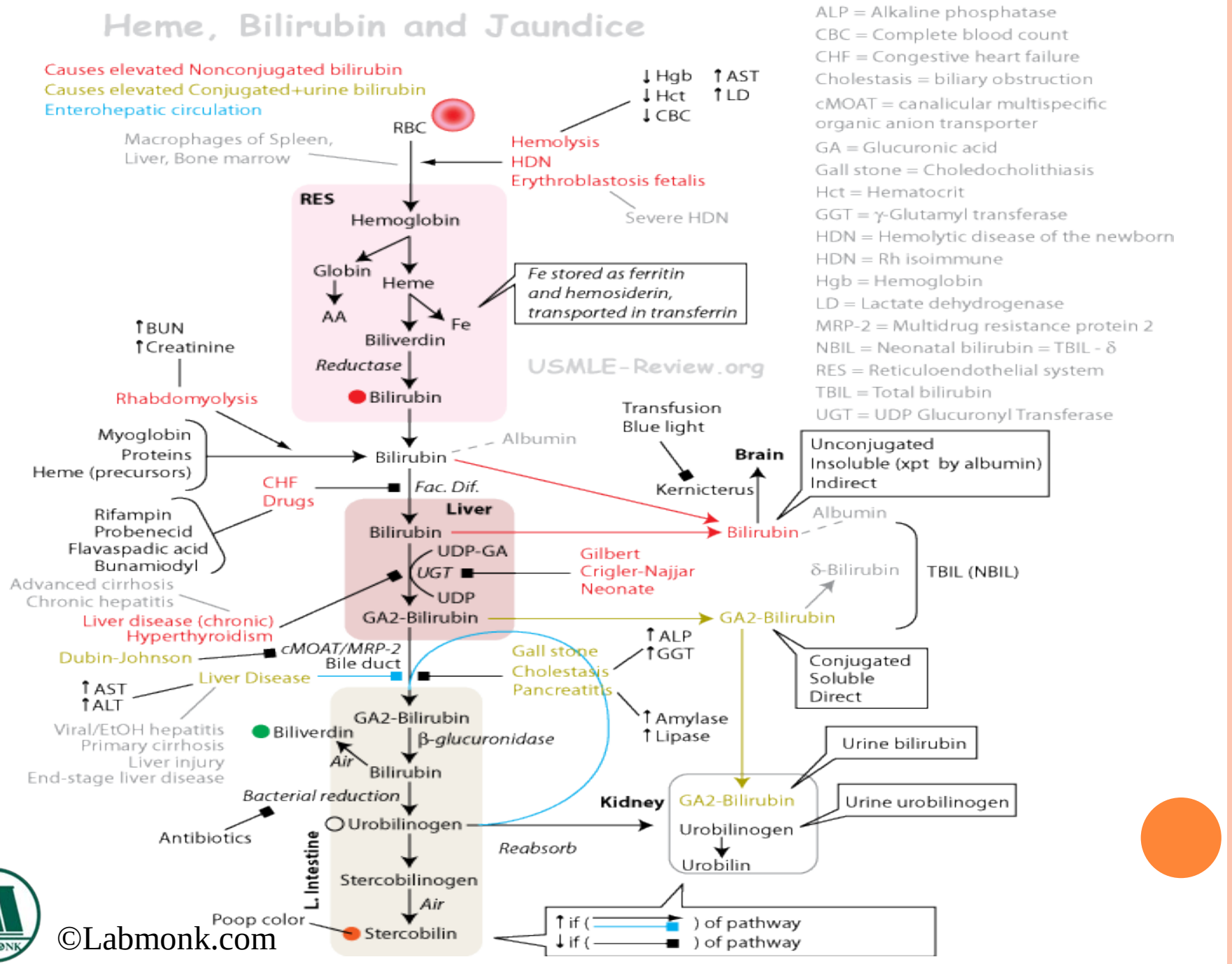
Enzymes:

- ALT(SGPT) – Marker enzyme for liver diseases
- AST(SGOT)
- Alkaline phospatase (ALP)
- Gama glutamyl transferase (GGT)
- 5’ – Nucleotidase

Special tests:

- Bile acid levels
- Blood ammonia
- α1- antitrypsin
- α1-Fetoprotein
- Hepatitis markers
- Immunoglobulins
- Ceruloplasmin
- Ferritin

Heme, Bilirubin and Jaundice



ALP = Alkaline phosphatase
CBC = Complete blood count
CHF = Congestive heart failure
Cholestasis = biliary obstruction
cMOAT = canalicular multispecific organic anion transporter
GA = Glucuronic acid
Gall stone = Choledocholithiasis
Hct = Hematocrit
GGT = γ-Glutamyl transferase
HDN = Hemolytic disease of the newborn
HDN = Rh isoimmune
Hgb = Hemoglobin
LD = Lactate dehydrogenase
MRP-2 = Multidrug resistance protein 2
NBIL = Neonatal bilirubin = TBIL - δ
RES = Reticuloendothelial system
TBIL = Total bilirubin
UGT = UDP Glucuronyl Transferase

Principle of the reaction (Bilirubin):

- The reagent is a mixture of equal volumes of sulfanilic acid in dilute HCl and sodium nitrite.

- That diazotised sulfanilic acid (the above mixture) reacts with bilirubin to form a purple coloured azobilirubin.

- **Direct Positive:**
- conjugated bilirubin gives a purple color immediately on addition of the reagent.

- **Indirect Positive:**
- Purple color develops only when the reagent and methanol are added.

- Unconjugated bilirubin gives color only when methanol is added.

Bile Salt Assay

- • Analysis done in fasting state
- • Assay done using chromatographic methods, HPLC

Sulphur Test-

- – Principle: BS ↓ surface tension of urine
- – Method: urine(10ml)+sulphur powder sprinkled
- →particles sink to bottom- BS present
- → particles float- BS absent

Determination of bile pigments

Harrison spot test :

- • urine sediment + Fouchet's reagent→
- No change in colour – BP absent
- change in colour to green – BP present
- Positive result graded as trace - ++++ as per
- intensity of colour of sediment

Urobilinogen determination

- • Freshly collected normal fasting urine sample- +ve reaction for urobilinogen
- • On air exposure oxidized to urobilin (pinkish brown)

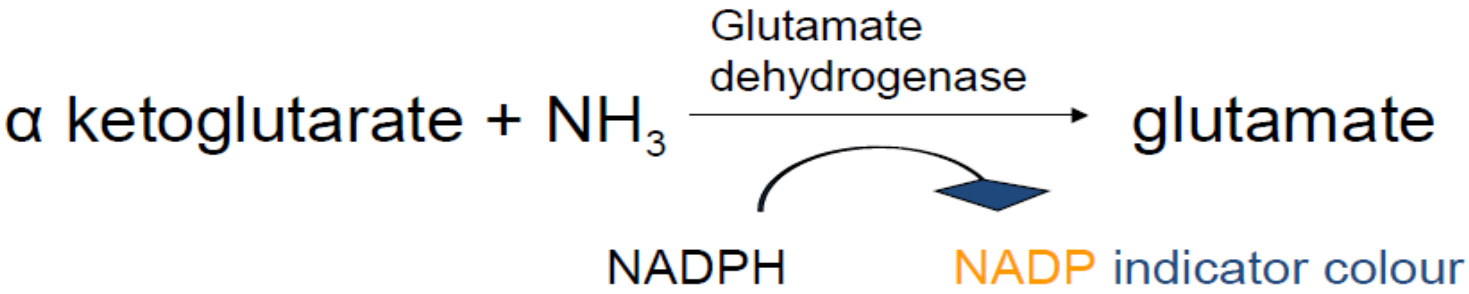
Test –

- – Urine + Ehrlich's reagent→ pale pink urobilinogen normal
- – cherry red- urobilinogen ↑↑↑
- – graded as per colour intensity

Assays for Ammonia

- • Arterial blood is prefered for assay
- • Specimen should be kept in ice water until separation of Cells from plasma

Enzyme assay



Dry slide method

- Alkaline pH buffers convert ammonium ions to ammonia gas
- – bromophenol blue - used indicator

Bilirubin in Urine:

- Normally bilirubin is absent in urine.
- Conjugated bilirubin being water soluble is excreted in urine in obstructive jaundice.
- This can be detected by Fouchet's test
- Urine urobilinogen - normally trace amounts is present.
- In obstructive jundice no urobilinogen is present in urine.

- because bilirubin cannot enter intestine.

Note: Presence of bilirubin in urine and absence of urobilinogen in urine is seen in obstructive jaundice.

- In hemolytic jaundice increased production of bilirubin causes increased formation of urobilinogen which appears in urine.

Note: Increased urobilinogen in urine and absence of bilirubin in urine is seen in hemolytic jaundice.

- **Fecal urobilinogen -** Normal about 300mg.
- Increased in Hemolytic jaundice in which color of feces is dark.
- In Obstructive jaundice urobilinogen is not excreted through feces and the color is the feces is pale.

JAUNDICE

- Clinical jaundice appears when bilirubin concentration is more than 3 mg/dl.

- Levels between 1 and 3 mg/dl is sub-clinical jaundice.

Classification of Jaundice:

Prehepatic

or

Hemolytic jaundice

or

Unconjugated hyperbilirubinemia

CAUSES :

- Increased production of unconjugated bilirubin from hemolysis - **sickle cell anemia**
- Rapid turnover of RBC - Neonate
- Physiological jaundice (Bilirubin 5mg/dl).
- Kernicterus Bilirubin >20mg/dl.
- Brain damage due to entry of bilirubin.
- No blood brain barrier.
- Decreased uptake of bilirubin by hepatocyte -
- Gilbert syndrome.
- Decreased conjugation - Neonatal Jaundice, drug inhibition , crigler – najjar syndrome, Hepatocellular dysfunction.

- Obstructive jaundice:

or

Post hepatic jaundice

or

Conjugated hyperbilirubinemia

- Decreased secretion of conjugated bilirubin into canaliculi - Hepatocellular disease, hepatitis.

- Decreased drainage - Intrahepatic obstruction by drugs , cirrhosis.

- Extra hepatic obstruction - stones , Carcinoma.

- **Hepatocellular jaundice**

- Acute hepatitis is usually caused by viral infections Hepatitis A, C, D, E. (or) by toxins

eg: paracetamol, Carbon tetrachloride etc.

Biochemical changes for the differential diagnosis of three types of jaundice

Parameter	Hemolytic jaundice (preheptic jaundice)	Obstructive jaundice (posthepatic jaundice)	Hepatic jaundice (Intrahepatic jaundice)
Serum bilirubin	Unconjugated bilirubin ↑	Conjugated bilirubin ↑	Both ↑
van den Bergh reaction	Indirect positive	Direct positive	Biphasic
Serum enzymes	ALT, AST and ALP →	ALP ↑↑, ALT and AST marginal ↑	ALT and AST ↑↑, ALP marginal ↑
Bilirubin in urine	Not excreted	Excreted	Excreted
Urobilinogen in urine	Excretion ↑	→ or ↓	→ or ↓

ALT : Alanine transaminase; AST : Aspartate transaminase; ALP : Alkaline phosphatase; ↑ : Increase; ↓ : Decrease; → : Normal.

Concentrations and Changes in Concentration of Bilirubin and its Metabolites in Healthy Persons and Those with Jaundice

	SERUM		URINE		
Condition	Total Bilirubin	Conjugated Bilirubin	Conjugated Bilirubin	Urobilinogen	Feces Pigment
Healthy	2 to 10 mg/L	0 to 2 mg/L	Negative	0.5 to 3.4 mg/day	Brown
Prehepatic jaundice	Increased	Normal	Negative	Increased	Normal
Hepatic jaundice					
Hepatocellular disease	Increased	Increased	Positive	Decreased (normal)	Light brown
Gilbert's disease	Increased	Normal	Negative	Decreased (normal)	Normal
Crigler-Najjar syndrome	Increased	Decreased	Negative	Decreased	Light brown
Dubin-Johnson syndrome	Increased	Increased	Positive	Decreased (normal)	Light brown
Posthepatic obstructive jaundice	Increased	Increased	Positive	Decreased	Light brown

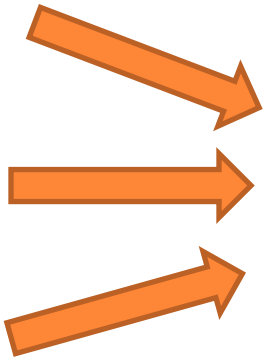
Serum albumin

- About 10 – 12 gm of albumin is synthesized in liver daily.
- Its estimation is very valuable in assessing chronic liver disease.
- Low serum albumin level is commonly observed in severe liver disease.

Protein Assays

Biuret method :

- • peptide backbone C=O+copper
- • Dye binding method :
- protein + Coomassie blue dye
- • Albumin + bromocresol green/ purple
- Normal total protein levels : 6-7.8 g/dl
- Albumin levels: 3.5- 5 g/dl



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Spectrophotometric quantitation

- **Prothrombin time** Normal 11 to 12 seconds

- PT is prolonged in severe parenchymal liver disease due to decreased synthesis of prothrombin.
- Vitamin K is required for synthesis of prothrombin.
- vitamin K deficiency can also lead to prolonged PT.

Note:

- If PT returns to normal after vitamin K injection it indicates that hepatocyte function is good.

Plasma proteins of diagnostic value in liver disease		
Protein	**Condition**	**Change in concentration**
albumin	chronic liver disease	↓
γ-globulins	cirrhosis, especially autoimmune	↑
α_1-antitrypsin	cirrhosis due to α_1-antitrypsin deficiency	↓
caeruloplasmin	Wilson's disease	↓
α-fetoprotein	primary hepatocellular carcinoma	greatly ↑
transferrin	haemochromatosis	normal but 100% saturated with iron

- **Transaminases:**
- ALT(SGPT) 3 to 15 IU/L
- AST(SGOT) 4 to 17 IU/L
- ALT is primarily localized to the liver. It is the marker enzyme of the liver.
- ALT is present in the cytosol of hepatocytes.
- AST is present in a wide variety of tissues like heart, liver, skeletal muscle, kidney, brain.
- AST is present both in the cytosol and mitochondria of the hepatocytes.

Liver contains both enzymes but more of ALT

- Estimation is very useful in assessing severity and prognosis of liver parenchymal disease especially infective hepatitis.

- Also very useful as screening test in outbreak of infective hepatitis.

Elevated ALT & AST

- Highly elevated > 20 times
- Viral hepatitis
- Drug or Toxin induced hepatic necrosis
- **Moderately elevated -** 3 to 20 times
- Chronic hepatitis
- Alcoholic hepatitis
- Auto immune hepatitis
- Acute biliary tract obstruction

Alkaline Phosphatase(ALP) - 3 to 13 KAU/dl

- A family of Zinc metallo enzymes, with a serine at the active center. They release inorganic phosphate from various organic phosphates.
- In the liver it is found in microvilli of bile canaliculi and on the sinusoidal surface of the hepatocytes.
- Other important sources of ALP is bone.
- ALP is highly elevated in obstructive jaundice and bone diseases like rickets.

Gamma -Glutamyl transpeptidase - Normal level 10 – 15U/L

- It is a membrane bound glycoprotein which catalyses the transfer of Gamma- glutamyl group to other peptides.
- Very useful in diagnosis of obstructive jaundice. (not elevated in bone diseases)
- It is a microsomal enzyme.
- Serum GGT is highly elevated in obstructive jaundice and alcoholic liver disease.
- This enzyme is an inducible enzyme.

5’ – Nucleotidase - Normal 2 to 15 U/L

- It is elevated in obstructive jaundice.
- Advantage of this enzyme is that it is not elevated in bone disease.

Test for assessing detoxification function of liver

- **Hippuric acid test:**

Principle :

- Hippuric acid is produced in the liver when benzoic acid combines with glycine.

Procedure :

- 6 gm of sodium benzoate is given to the patient.
- Urine is collected upto 4 hours
- Hippuric acid excreted in urine is estimated.
- 6 gm of sodium benzoate forms 7.5 gm of hippuric acid.
- 60% of Sodium benzoate (4.5gm of Hippuric acid) is excreted in normals.
- Decreased hippuric acid excretion < 3gm indicates hepatic damage.

Test \ Condition	Acute hepatitis	Chronic hepatitis	Cirrhosis	Cholestasis	Malignancy and infiltrations
Bilirubin	N to ↑↑	N to ↑	N to ↑	↑ to ↑↑↑	N
Aminotransferases	↑↑↑	↑	N to ↑	N to ↑	N to ↑
Alkaline phosphatase	N to ↑	N§	N to ↑↑	↑↑↑	↑↑
Albumin	N	N to ↓	N to ↓	N	N to ↓
γ-Globulins	N	↑	↑	N	N
Prothrombin time	N to ↑*	N to ↑	N to ↑*	N to ↑†	N

Patterns of abnormalities of simple liver function tests in various liver diseases. The severity of the abnormalities is dependent on the degree of liver damage and its effect on liver function.

N = Normal

* Not corrected by parenteral vitamin K. § May be increased if cirrhosis is present.

ected by parenteral vitamin K.

THANK YOU

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