INDICATION

1. Jaundice
2. Alcoholic liver disease
3. Secondary metastasis to liver
4. Undiagnosed chronic illness
5. Coagulation disorder
6. Before administration of certain drugs
7. Annual check up of diabetes mellitus

In liver diseases-

- To detect presence of liver disease
- Distinguish among different types of liver disorders
- Gauge the extent of known liver disease
- Follow the response to treatment

- Some tests are associated with FUNCTIONALITY (e.g. PT/INR, Albumin, Bilirubin),
- some with CELLULAR INTEGRITY (e.g. transaminases) and
- some with conditions linked to BILIARY TRACT (GGT and ALP)
- …..and hence liver biochemical tests are classified into following groups:

A. BIOCHEMICAL CLASSIFICATION

1. TESTS BASED ON LIVER EXCRETORY FUNCTION

a) Serum bilirubin- total
- conjugated
- unconjugated

b) Urine – bile pigment
- bile salt
- urobilinogen

2. Liver enzymes

I. AST

II. ALT

III. ALP

IV. GGT

3. TESTS BASED ON LIVER SYNTHETIC FUNCTION

I. SERUM TOTAL PROTEIN
II. SERUM ALBUMIN
III. ALBUMIN GLOBULIN RATIO
IV. PROTHROMBIN TIME

4. SPECIAL TESTS (TESTS DONE IN SPECIAL SITUATIONS) ARE:

i) Ceruloplasmin
ii) Transferrin
iii) α-1Anti Trypsin
iv) Alpha Feto Protein

CLINICAL CLASSIFICATION

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Group I: Markers of liver dysfunction

- Serum bilirubin: total = conjugated+ Unconjugated
- Urine: urobilinogen, bile salts and bilirubin
- Total protein, serum albumin, globulin and albumin/globulin ratio
- Prothrombin Time

Group II: Markers of hepatocellular injury

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

Group III: Markers of cholestasis

- Alkaline phosphatase (ALP)
- γ-glutamyltransferase (GGT)

Group IV :Special tests (tests done in special situations) are:

- Ceruloplasmin
- Transferrin
- α-1Anti Trypsin
- Alpha Feto Protein
- Blood ammonia
- Galactose tolerance test
- procollagen III peptide
- Bromsulphthalein test
- Anti-mitochondrial antibody test
- Biochemical test for liver fibrosis

1. serum bilirubin:

- Normally, a small amount of bilirubin circulates in the blood.
- Serum bilirubin is considered a true test of liver function, as it reflects the liver's ability to take up, process, and secrete bilirubin into the bile

A. Indirect bilirubin (normal value = 0.3 - 1.2 mg/dl)

B. Direct bilirubin (normal value ≤ 0.4 mg/dl)

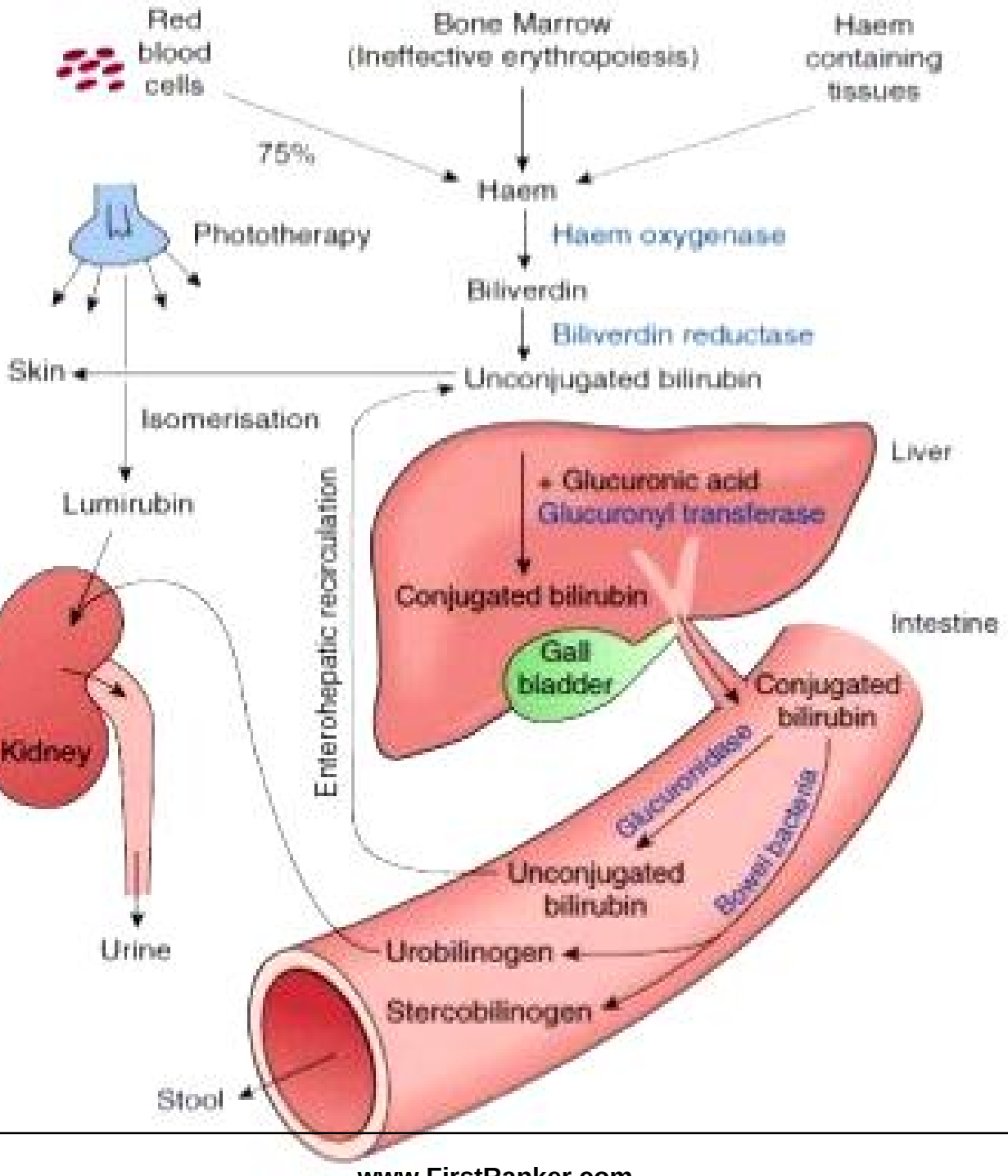
C. Total bilirubin (normal value =0.3-1.2 mg/dl)

- If the plasma bilirubin level exceeds 1.2mg/dl, the condition is called hyperbilirubinemia.
- Levels between 1.2 & 2.5 mg/dl are indicative of latent jaundice.
- When the bilirubin level exceeds 2.5 mg/dl, it diffuses into tissues producing yellowish discoloration of sclera, conjunctiva, skin & mucous membrane resulting in jaundice.

latent jaundice 1.2-2.5 mg/dl

Clinical jaundice > 2.5 mg/dl

- Icterus is the Greek term for jaundice.



- It’s level confirms jaundice, and used to assess the prognosis.
- It’s level represents the balance between input from production and hepatic removal of the pigment.
- Unconjugated hyperbilirubinemia is due to overproduction or impaired uptake or conjugation of bilirubin.
- Conjugated hyperbilirubinemia is due to decreased excretion or backward leakage of the pigment.

VAN DEN BERGH REACTION:

- Normal serum gives a negative van den bergh reaction.
- PRINCIPLE OF THE REACTION:

  The reagent is a mixture of equal volumes of sulfanilic acid in dilute HCl and sodium nitrite. (DIAZOTISED SULFANILIC ACID)

  That diazotised sulfanilic acid 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.

- BiPhasic: Purple color develops on addition of reagent. Addition of methanol intensifies the color.

  Elevation of both unconjugated and conjugated bilirubin

Indirect Positive----- Hemolytic jaundice

Direct Positive ----- Obstructive jaundice

Biphasic ----- Hepatic jaundice

Depending upon the etiology of hepatitis :

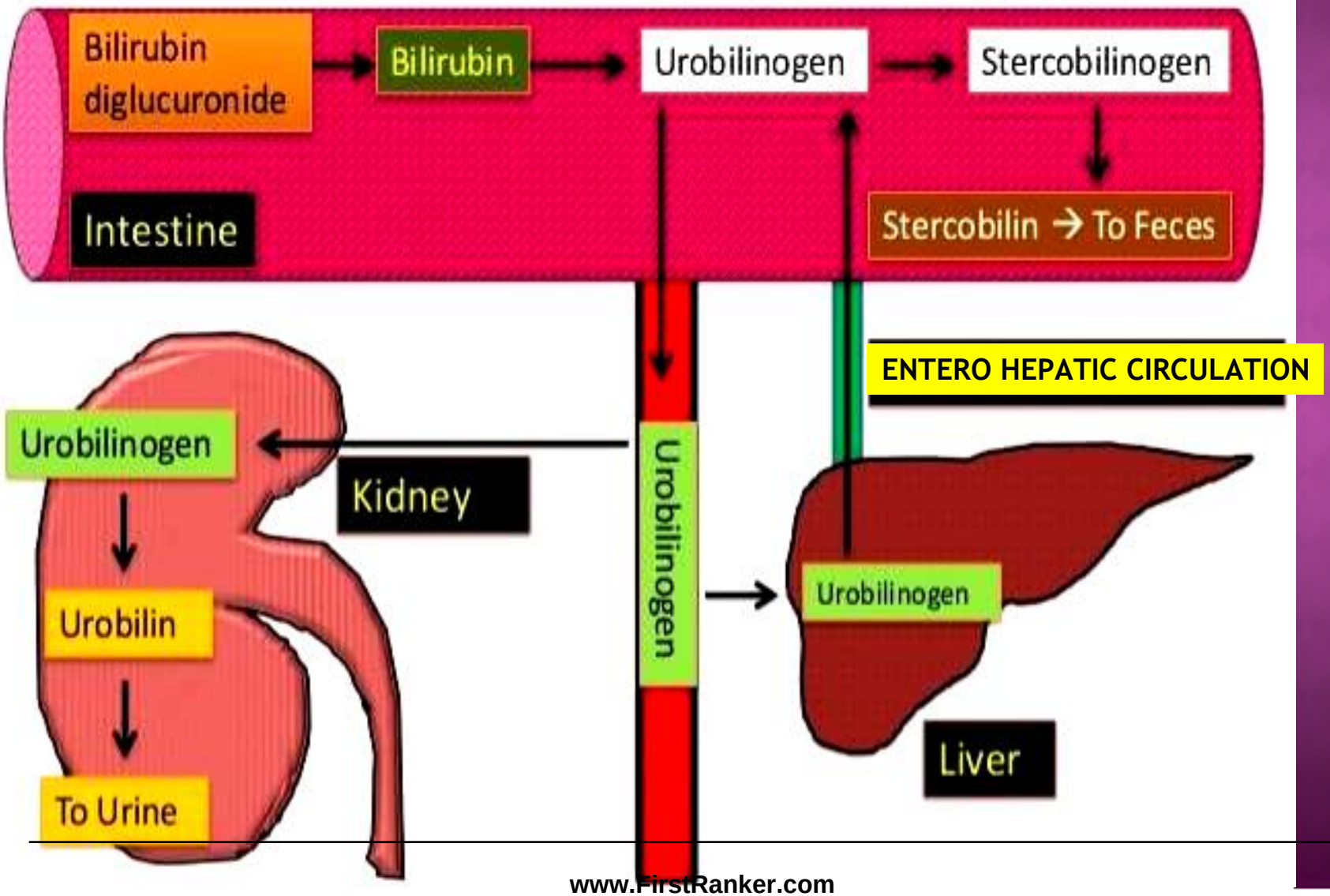
Conjugated hyperbilirubinemia:-

- Viral hepatitis
- Alcoholic hepatitis
- Toxic hepatitis
- Active cirrhosis
- Genetic disease– Dubin johnson syndrome and rotor's syndrome

Unconjugated hyperbilirubinemia:-

- crigler najjar and gilbert's syndrome
- physiological jaundice of newborn

Fate of Bilirubin



2.URINE UROBILINOGEN

- UBG is formed in terminal ileum and colon from conjugated Bb by Clostridium ramosum, helped by E.coli.
- UBG excreted in stool is called stercobilinogen. It is converted by colonic bacteria to stercobilin which imparts the normal brown colour of stools.
- Hence in cholestatic jaundice stools are pale as Bb can not reach the gut and hence stercobilin is not formed.
- About 20% of UBG is reabsorbed and undergoes enterohepatic circulation.
- Increase in UBG in urine is found in hepatitis as damaged hepatocytes are not able to reexcrete the UBG absorbed from gut. It is thus a good index of hepatocellular dysfunction, often when other tests are normal.

- Urine UBG is increased in :

  1)hepatitis 2)malignant disease of liver

  3)cirrhosis 4)hemolytic anaemia

- UBG is absent in :

  1) complete biliary obstruction

  2) severe bilirubin glucoronyl transferase deficiency as seen in CN syndrome type I.

3.URINE BILE SALT

- Bile salts are formed in the liver from cholesterol
- They are excreted in bile
- Facilitate absorption of fat from intestine
- Constitute a substantial amount of bile in bilirubin excretion and can be used in diagnosing cholestasis
- Primary bile salts – cholate and chenodeoxycholate are produced in liver

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  Metabolised by bacteria in intestine

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  Produces secondary bile salts – lithocholate, deoxycholate and ursodeoxycholate

- In cirrhosis – reduced ratio of primary to secondary bile salts
- In cholestasis – as secondary bile salts are not formed – so increased ratio of primary to secondary bile salts.

In normal condition – renal excretion of bile salts is negligible

In Hepatocellular jaundice, swollen liver cells compress biliary canaliculi

Hence, there is intrahepatic obstruction of biliary canaliculi

Bile salts cannot reach intestine, they are regurgitated from liver into systemic circulation and appear in urine

4.URINE BILIRUBIN

- Bilirubin is not normally present in urine and faeces since bacteria in intestine reduce it to urobilinogen.
- The kidneys do not filter unconjugated bilirubin because of its avid binding to albumin.
- Conjugated bilirubin can pass through glomerular filter.
- Conjugated bilirubin in serum is raised in hepatocellular and obstructive jaundice.
- Therefore, bilirubin is present in urine in hepatocellular and obstructive jaundice
- Bilirubin in the urine may be detected even before clinical jaundice is noted.
- Recovering from jaundice urine bilirubin clears prior to Sr bilirubin

Specimen	Test/Compound	Prehepatic jaundice	Hepatic jaundice	Posthepatic jaundice
Blood	Unconjugated bilirubin *(van den Bergh indirect test)*	Elevated	Elevated	Normal
	Conjugated bilirubin *(van den Bergh direct test)*	Normal	Excretion is rate limiting. It is the first sign of impaired activity. In the early phase, it is increased.	Elevated
	Alkaline phosphatase (40–125 U/L)	Normal	2–3 times increased	10–12 times increased
Urine	Bile salt *(Hay's test)*	Absent	Absent	Present
	Conjugated bilirubin *(Fouchet's test)*	Absent	Present	Present
	Urobilinogens *(Ehrlich's test)*	Elevated	Increased in early phases; later decreased because production is low. Earliest manifestation of recovery is presence of urobilinogen in urine	Absent
Feces	Urobilins	Elevated	Normal or decreased	Clay coloured

5.DETERMINATION OF TOTAL PROTEIN ,ALBUMIN, GLOBULIN & A:G RATIO

- This yields most useful information in chronic liver diseases.
- Liver is the sole site for synthesis of most plasma proteins except immunoglobulin (gamma globulins)
- Normal value:

total serum proteins =6.0 to 8.0 gm/dl,

serum albumin (A) = 3.5 to 5.0 gm/dl.

serum globulin(G)= 2.5-3.5 gm/dl

A:G RATIO=1.5:1 to 2.5:1

- Serum albumin comprises 60% of all plasma proteins
- Half-life of albumin is 14 to 21 days makes it unreliable in acute liver failure

- In infectious hepatitis:
  quantitative estimations of albumin and globulin may give normal results in the early stages. qualitative changes may be present, in early stage rise in ß -globulins and in later stages γ-globulins shows rise.

- cirrhosis or parenchymal liver disease:
  The albumin is grossly decreased and the globulins are often increased, so that A:G ratio is reversed, is characteristically seen in cirrhosis of liver.

6.PROTHROMBIN TIME

- With the exception of F-VIII , all other coagulation factors are synthesized in liver
- Half life ranges from 6hrs for F-VII to 5 days for fibrinogen
- So their measurement is the single best measure of hepatic synthetic function
- Measured by Prothrombin time

- Prothrombin —vitamin K→ thrombin

- •Marked increase in PT >5secs above the control and not corrected by Vit K administration – is a poor prognostic sign in acute viral hepatitis and other acute and chronic liver disease

7.SERUM TRANSAMINASE

- ALANINE TRANSAMINASE (ALT)
- ASPARTATE TRANSAMINASE (AST)

Liver enzymes are important markers of hepatocellular damage & severity of liver diseases.

AST

Heart, skeletal muscle, brain, pancreas, lung, RBC and kidney.

20% cytosolic and 80 % mitochondrial

serum half life of 17 hrs.

ALT

ALT is more specific for liver

Low concentrations in kidney and skeletal muscles

serum half life of 47 hrs

DE RITI'S RATIO: THE AST:ALT RATIO

- Normal ratio is 0.7 to 1.4.
- In alcoholic hepatitis, the AST:ALT ratio is always ≥ 2:1.
- The ratio is usually <1 in patients with acute and chronic non- alcoholic hepatitis.

- Most marked elevations of ALT and AST (>15 times normal) are seen in
  - acute viral hepatitis
  - toxin-induced hepatocellular damage (e.g. carbon tetrachloride and
  - centrilobular necrosis due to ischemia (congestive cardiac failure).

Moderate elevations (5-15 times) occur in

- Chronic hepatitis,
- autoimmune hepatitis
- alcoholic hepatitis
- acute biliary tract obstruction
- drug-induced hepatitis

- Mild elevations (1-3 times) are seen in

- cirrhosis,
- nonalcoholic steatosis
- cholestasis.

Diagnostic value of transaminases

- The first laboratory abnormality detected in early phase of viral hepatitis is elevated transaminases
- In anicteric hepatitis and inapparent hepatitis the only biochemical abnormality may be an elevated ALT or AST.
- Fluctuating levels of transaminases may be seen in hepatitis C infection .
- In hepatitis, elevation of transaminases precedes that of bilirubin by about one week.
- During recovery phase of viral hepatitis, there is a steady fall in level of transaminases.

8. Alkaline phosphatase

- Alkaline phosphatase (ALP) is synthesized in liver, bones, intestine and placenta
- In liver, ALP is synthesized by parenchymal cells as well as epithelial cells of biliary canaliculi

- Serum ALP is mildly raised in viral hepatitis due to necrosis of parenchymal cells
- A marked elevation in serum ALP occurs in obstructive jaundice

- The rise is due to increased synthesis of ALP caused by irritation of epithelial cells of biliary canaliculi

9.GAMMA GLUTAMYL TRANSPEPTIDASE (γ GGT)

- It is synthesized by epithelium of small bile ductules and hepatocytes
- GGT levels are higher in biliary tract disease and cholestasis than in hepatocellular disease.
- Rise in serum GGT is a sensitive indicator of alcoholic hepatitis
- An elevated GGT is used to confirm that a raised ALP is of hepatobiliary origin. Hence it is a more sensitive marker compared to ALP.

10.SPECIAL TESTS

A.Ceruloplasmin

Normal plasma levels - 0.2-0.4g/L

- Acute phase protein

Decreased in multiple conditions

1. Wilson's disease (Hepatolenticular degeneration)
2. Menkes disease
3. Aceruloplasminemia
4. Copper deficiency

B. Blood ammonia

- In advanced liver disease, liver may fail to convert ammonia into urea

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Increased blood ammonia

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can cause hepatic encephalopathy

- Measurement of blood ammonia helps in its diagnosis and monitoring.

C. ALPHA -1 ANTITRYPSIN (α-1 AT)

- Major α-1 globulin protein
- Responsible for 90% of plasma tryptic inhibitory capacity
- α-1 AT deficiency is a major cause of chronic liver disease in children
- Less commonly, of chronic liver disease presenting in adulthood.

D. TRANSFERRIN

- An iron transfer protein (Normal-30-40% saturated)
- Its saturation is used as a screening test for Hemochromatosis (>60% saturated)
- Decreased saturation is found in cirrhosis and malnutrition.

E. ALPHA FETO PROTEIN

- Normal component of fetal blood but disappears few week after birth.
- Mild elevation is seen in cirrhosis, acute and chronic hepatitis
- Higher concentration is seen in hepatocellular carcinoma.

F. GALACTOSE TOLERANCE TEST

- Galactose is almost exclusively metabolized by the liver.
- The liver function can be assessed by measuring the utilization of galactose.
- The subject is given intravenous administration of galactose (about 300 mg/kg body weight).
- Blood is drawn at 10 minute intervals for the next 2 hours & galactose estimated.
- In the normal individuals, the half-life of galactose is about 10-15 minutes.
- This is markedly elevated in hepatocellular damage (infective hepatitis, cirrhosis).

G. BROMOSULPHTHALEIN DYE TEST

- Bromosulphthalein is a dye used to assess the excretory function of liver.
- It is a non-toxic compound & almost exclusively excreted by the liver (through bile).
- BSP is administered intravenously (5 mg/kg body weight) & its serum concentration is measured at 45 min & at 2 hrs.
- In normal individuals, <5% of the dye is retained at the end of 45 min.
- Any impairment in liver function causes an increased retention of the dye.
- Increased plasma retention can result from decreased excretory rate as seen in Dubin Johnson Syndrome.

H. Mitochondrial Antibodies Test

- The presence of these antibodies can indicate
  - primary biliary cirrhosis,
  - chronic active hepatitis, and
  - certain other autoimmune disorders.

11.TEST TO DETECT HEPATIC FIBROSIS

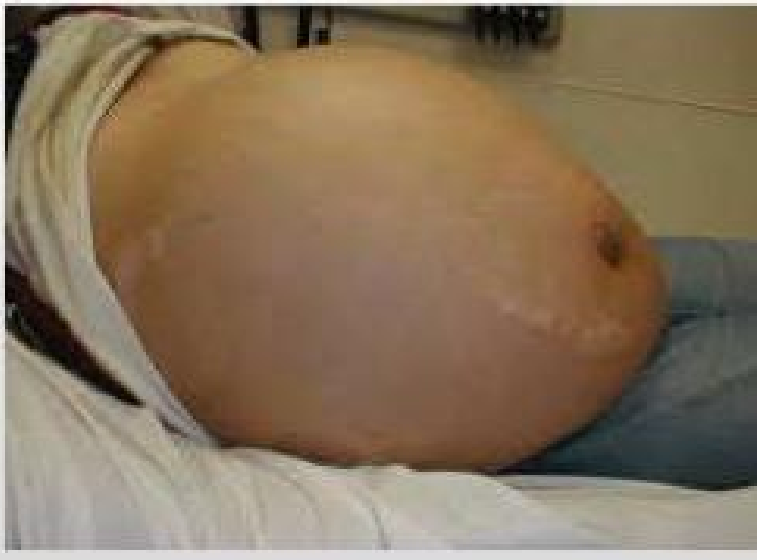
- liver biopsy is the standard for the assessment of hepatic fibrosis.
- Need has arrived to go for non invasive tests.
- Single serum biochemical markers that potentially reflect the activity level of hepatic fibrogenesis - Hyaluronan.
- A fasting hyaluronan level greater than 100 mg/L (sensitivity83% & specificity78%)for the detection of cirrhosis in patients with a variety of chronic liver diseases like chronic hepatitis C, chronic hepatitis B, alcoholic liver disease, and nonalcoholic steatohepatitis

LAST BUT NOT LEAST…

ASCITES FLUID

THE SPECIAL "LFT"

Ascites (A-sigh-teas) is the accumulation of an excessive (larger than normal) amount of fluid in the abdominal cavity.



PARACENTESIS

- **Tests**
  - 4 C's: Cells, Culture, Chemistry, Cytology
  - Cell count and differential, gram stain, culture, albumin, total protein, glucose, LDH, cytology
  - Optional: amylase, bilirubin, Cr, TG, AFB cx + adenosine deaminase
- **Calculation of SAAG**
  - SAAG = [Serum albumin] – [Ascites albumin]
- **What does the SAAG indicate?**
  - If ≥ 1.1 g/dL, portal HTN is very likely (~97% accurate[1])
  - If < 1.1 g/dL, portal HTN is unlikely.