Liver Function Test

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Liver Function Test

- The most commonly available laboratory test for liver disease include measurement of plasma levels of:
- 1.Bilirubin excretory function
- 2.Transaminases(ALT&AST) cell damage
- 3.Alkaline phosphatase cholestasis
- 4.Albumin and prothrombin time synthetic function

The major Functions of the liver are:

1) Carbohydrate metabolism .

A) Glugconeogenesis.

B) Glycogen synthesis & metabolism.

2) Fat metabolism.

A)Fatty acid synthesis

B)cholesterol synthesis &excretion

C)Lipoprotein synthesis..

D) Ketogensis .(converting) The fatty acid to ketone bodies.

3) Protein metabolism.

A) synthesis of plasma proteins.

B) Urea synthesis.

4) Hormone metabolism.

Metabolism , conjugation &excretion of steroidal &poly peptide hormones .

5) Drugs &foreign compounds; metabolism& excretion of drugs.

6) Liver is a good part in storage of:

a)glycogen

b)VIT . A

B)VIT . B12

C)Iron.

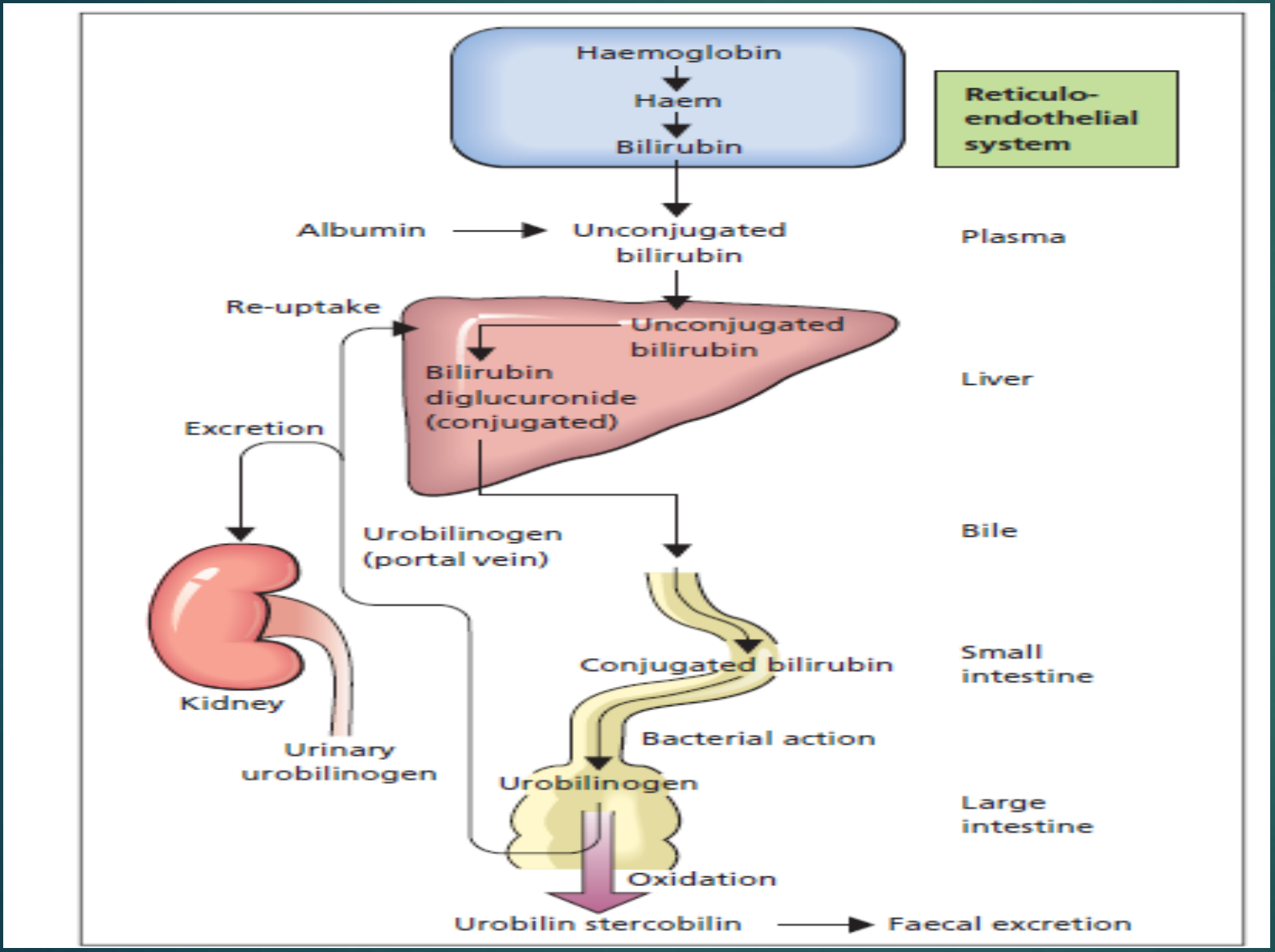
7) Also liver plays a good part in metabolism & secretion of Bilirubin.

- There fore ,any damage to the liver organ may affect any of the above functions.

Bilirubin

- **Metabolism:**
- RBC live approximately 120days and are then destroyed.
- Bilirubin is a waste product derived from hemoglobin destruction(from porphyrin rings).
- The route of excretion is through the biliary tract.
- The formation and excretory pathways is given in figure 1.

Figure 1:Metabolism of bilirubin.



Important note about the figure:

- 1.Bile pigments originate from RE cells(Kupffer cells in liver or other RE cells where erythrocytes are destroyed).The globin is broken down to amino acids . Which go to the amino acid pool. Iron enters the body stores to be reused. The protoporphyrin ring is opened to form bilivirdin. The biliverdin is reused to form bilirubin within RE cells.
- RBC destroyed → globin chains – amino acid pools.
  → Fe –iron stores.
  → porphyrin rings.

2.The bilirubin formed in RE cells(other than kupffer cells) is transported to the liver sinusoidal spaces via a bilirubin –albumin complex. In the liver the albumin is separated from bilirubin may then be used again for further transport.

- 3.In the liver parenchymal cells. Bilirubin is conjugated with glucuronide through a series of enzymatic reaction ,the most important one is UDP-glucuronyl transferase.
- Before conjugation ,bilirubin is not water soluble and is called indirect on un conjugated bilirubin. After conjugation ,bilirubin is water soluble and is called direct or conjugated bilirubin. conjugated bilirubin can be excreted in urine.
- 4. The bilirubin –diglucoronide is excreted into the bile and passes in to the small intestine where it is reduced by an aerobic bacterial enzymes. The reduction products consist of mesobilirubinogen (all are collectively known as urobilinogen).
- 5.Aportion of the urobilinogen is reabsorbed from the intestinal tract and returned to the liver via the enterohepatic portal system. Normally the liver removed all but a small portion of the recycled urobilinogen and re excreted it via the bile .urobilinogen is water soluble.

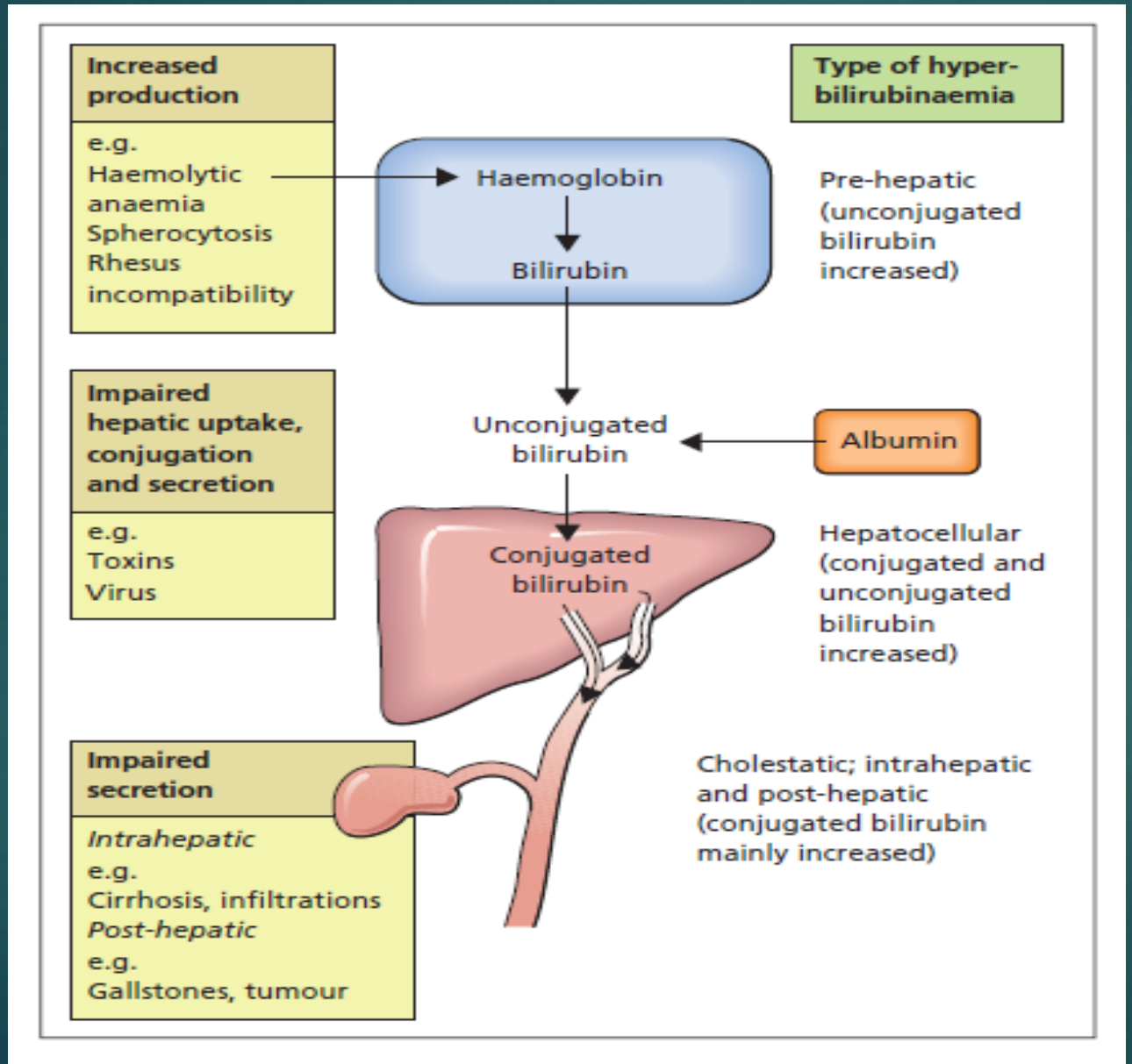
- 6.The urobilinogen remaining in the intestine is oxidized to urobilin. Which is colored and gives feces its brown color.
- There are two forms of bilirubin in the body :indirect or un conjugated bilirubin, which is protein bound and direct or conjugated bilirubin ,which is circulate freely in the blood until it reaches the liver ,Where it is conjugated with glucuronides and then excreted into the bile.

Jaundice

- Is the clinical manifestation of raised plasma bilirubin level, when the bilirubin in the blood is excessive it deposits in the tissue ,which then become yellow. The condition is known as jaundice or icterus. Levels of total serum bilirubin above 2.5-3.0 mg/dl usually produce jaundice.
- In healthy individuals all or most of the total bilirubin is unconjugated , resulting from daily destruction of RBC.
- Jaundice may be caused by 1.production of more bilirubin than the normal liver can excrete(this type of jaundice occur in hemolytic disease),2.failure of the damaged liver to excrete the bilirubin produced in normal amounts (this type of jaundice may occur in hepatitis or in liver disease associated with hepatotoxins), 3.obstruction of the excretory ducts of the liver.

- The obstruction may occur in the liver, and is then called intrahepatic cholestasis , or the obstruction may occur in the extrahepatic biliary tree and is called extrahepatic cholestasis or post hepatic obstruction .Intrahepatic obstruction occurs when there is defective transport of conjugated bilirubin into bile canaliculi.
- This can occur as a result of inflammation or swelling of liver cells ,which then block excretory ducts in the liver . Extrahepatic obstruction may occur due to stones ,tumor or stricture causing obstruction of the biliary tree.

Figure 2:classification of jaundice



Classification of Jaundice

- A laboratory classification of jaundice depend on fractionated bilirubin is given below:
- 1-Unconjugated :I this type of jaundice at least 80% of serum bilirubin is unconjugated or in direct.
- A-Pre hepatic: this occur due to increased production of bilirubin secondary to increased destruction of RBC.
- increased destruction of RBC occur in *hemolytic anemia and *neonatal jaundice due to Rh or ABO incompatibility.

- B-Hepatic: this occur due to defective removal of bilirubin from sinusoidal blood by the liver parenchymal cells or from sinusoidal blood by the liver parenchymal cells or due to a conjugated defect in the liver:
- *Pre conjugation transport failure: occur in Gilberts disease, is a familial type of non hemolytic jaundice.it is relatively common and a symptomatic . The transport of bilirubin into liver cells from the sinusoidal space is impaired the plasma bilirubin fluctuates and increased during illness.
- *Conjugation failure of the liver;

- Neonatal physiological jaundice is a common type of jaundice found in the newborn. This type of jaundice is due to an inefficient or immature fetal liver, the liver is unable to conjugate all of the bilirubin produced because of deficient enzymes. As the liver mature ,enzyme function improves and jaundice disappear in few days .Neonatal physiological jaundice treated with phototherapy.
- Crigler –Najjar disease is second type of jaundice caused by conjugation failure ,this type caused by deficiency of conjugating enzyme system in the liver .

2.Conjugated

- In the Conjugated type the percentage of Conjugated or direct bilirubin to total bilirubin is much higher than in the unconjugated type, the percentage of conjugated bilirubin may reach 40-50% or higher .
- A- Hepatic:
- *Hepatocellular: diffuse hepatocellular damage or necrosis causes the conjugated bilirubin to regurgitate into sinusoidal blood. The damaged liver cell is in capable of normal function and cannot efficiently removes the bilirubin normally produced; this derangement results in increased unconjugated bilirubin also. Hepatocellular damage may result from (a)viral hepatitis;(b)toxic hepatitis;(c) cirrhosis.

- *Hepato canalicular: Intrahepatic obstruction causes defective transport of conjugated bilirubin in to the bile canaliculi ,intrahepatic obstruction may occur due to (a) viral hepatitis ;(b)drugs;(c) Dubin –Johnson disease(genetic defect result in post conjugation transport failure , the conjugated bilirubin can not be transported in to bile canaliculi).
- B- Post hepatic : is caused by obstruction of the common bile duct. The obstruction may be caused by (a)stones;(b)stricture or spasm and (neoplasm).

Bilirubin Test

- Photometric test for direct (D) and total (T)bilirubin.
- Name of the method :Modified Jen drassik/Grof method.
- Principle of the method:
- Bilirubin react with diazotized sulfanilic acid (DSA) to form red azo dye.
- The absorbance of this dye at 546 nm is directly proportional to the bilirubin conc.in the sample. Water soluble bilirubin glucuronides react directly with DSA whereas the albumin conjugated indirect bilirubin will only react with DSA in the presence of an accelerator.

Specimen

- A. Recommended specimen is 100 µl of serum or heparinized plasma. Collect specimens by standard venipuncture technique. Handle specimens in stoppered containers to avoid contamination and evaporation. Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum-based products. Discard contaminated materials with infectious waste.
- B. Protect specimens from light and analyse as soon as possible after collection. Direct exposure to sunlight is reported to cause as much as 50% loss of bilirubin in one hour, especially when the specimen is kept in a capillary tube. Exposure to normal room light can result in a significant loss of serum bilirubin after 2 to 3 hours.

- C. If analysis is not performed immediately; samples can be refrigerated for up to 24 hours at (2–5) °C.
- D. Samples that come in contact with alcohol from sterile wipes may become haemolysed, which will increase the value of bilirubin.
- E. If concentration is greater than the analyzer range, dilute with 5 volumes of normal saline and reanalyze. Multiply the result by 6 to obtain the original total bilirubin concentration.

Reagents and materials

A. Spectrophotometer and bilirubimnometer for neonate.

B. Centrifuge

C. Gel tube (clot activator)

D. Reagents

- All reagents are ready to use.
- Wave length is 546 nm.

Reagent component:

- R1 (TBR) — Sulphanilic acid, sodium benzoate, Hydrochloric acid ,caffeine(accelerator).
- R2 (TNR) — Sodium nitrate for determination of total bilirubin
- R3 (DBR) — Sulphanilic acid Hydrochloric acid
- R4 (DNR) — Sodium nitrate for direct bilirubin

Procedure: For total bilirubin

	Sample Blank	Sample
TBR(R1)	1ml	1ml
TNR(R2)		1drop
Mix and incubate 5min.at (15-25 c)		
Serum	100µl	100µl
Mix and incubate at room temperature for 10-30min.Measure the absorbance of sample against sample blank		

Procedure of direct

	Sample blank	Sample
DBR	1ml	1ml
DNR		1 drop
Mix thoroughly ,add sample within 2min		
Sample	100µl	100µl
Mix and incubate for 5 min. read absorbance .		

Calculation

- Total bilirubin = Absorbance * 13
- Direct bilirubin = Absorbance * 13
- Normal Value:
- For total:
- At birth up to 5mg/dl
- 5days up to 12
- 1month up to 1.5
- Adult up to 1.1
- For direct
- Adult 0.25 mg/dl