



College of Pharmacy

Fifth Stage

Clinical Chemistry

Dr. Maytham Ahmed

Lecture 2

Liver Function Tests

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1. Total serum bilirubin (TSB), direct (conjugated) and indirect (unconjugated) bilirubin.
2. Albumin and prothrombin time.
3. Liver enzymes include:
Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), γ - Glutamyl transferase (GGT) and Lactate dehydrogenase (LD).

1. Bilirubin metabolism

Reticulo-endothelial system is mainly in liver, spleen and bone marrow. About 80% of bilirubin formed from heme each day arise from red blood cells. The remaining 20% comes from red cell precursors destroyed in the bone marrow (ineffective erythropoiesis), and from other heme proteins such as myoglobin, cytochromes, catalase and peroxidase.

Hemoglobin is broken down to **globin and heme**. Globin go to protein pool. **Heme** is broken down to **iron** (go to iron pool) and **biliverdin** by heme oxygenase, then biliverdin reduced by biliverdin reductase to produce bilirubin. **Bilirubin is soluble in lipid solvents but almost insoluble in water**. These characteristics enable it to cross cell membrane readily, but special mechanisms are needed to make it water-soluble for carriage in plasma by **protein-binding mainly to albumin forming indirect or unconjugated bilirubin**. In this form, it does not readily enter most tissues, nor it is filtered at the glomerulus.

The bilirubin-albumin complex appears to be associated by receptors on the plasma membrane of the hepatocytes, bilirubin taken up by a specific carrier (facilitated diffusion), leaving albumin in the plasma. **Conjugation of bilirubin within the hepatocytes makes it water-soluble.** The enzyme is Bilirubin-UDP-glucuronyl transferase forms **bilirubin-diglucuronide (direct or conjugated bilirubin)**. Then it secreted into bile and then to intestine. Bilirubin diglucuronide is degraded by bacterial action, mainly in the colon, being deconjugated and then converted into a mixture of compounds collectively termed **urobilinogen (stercobilinogen)**.

Urobilinogen is water soluble, mostly excreted in the feces but a small percentage (20%) is reabsorbed and then mostly re-excreted by the liver. After excretion, **urobilinogen (colorless)** is oxidized to **stercobilin which is brown** and gives stools its color. Some of the reabsorbed **urobilinogen** passes through the liver into the systemic circulation and is then excreted in the urine (**urobilin**) gives the urine its yellow color.

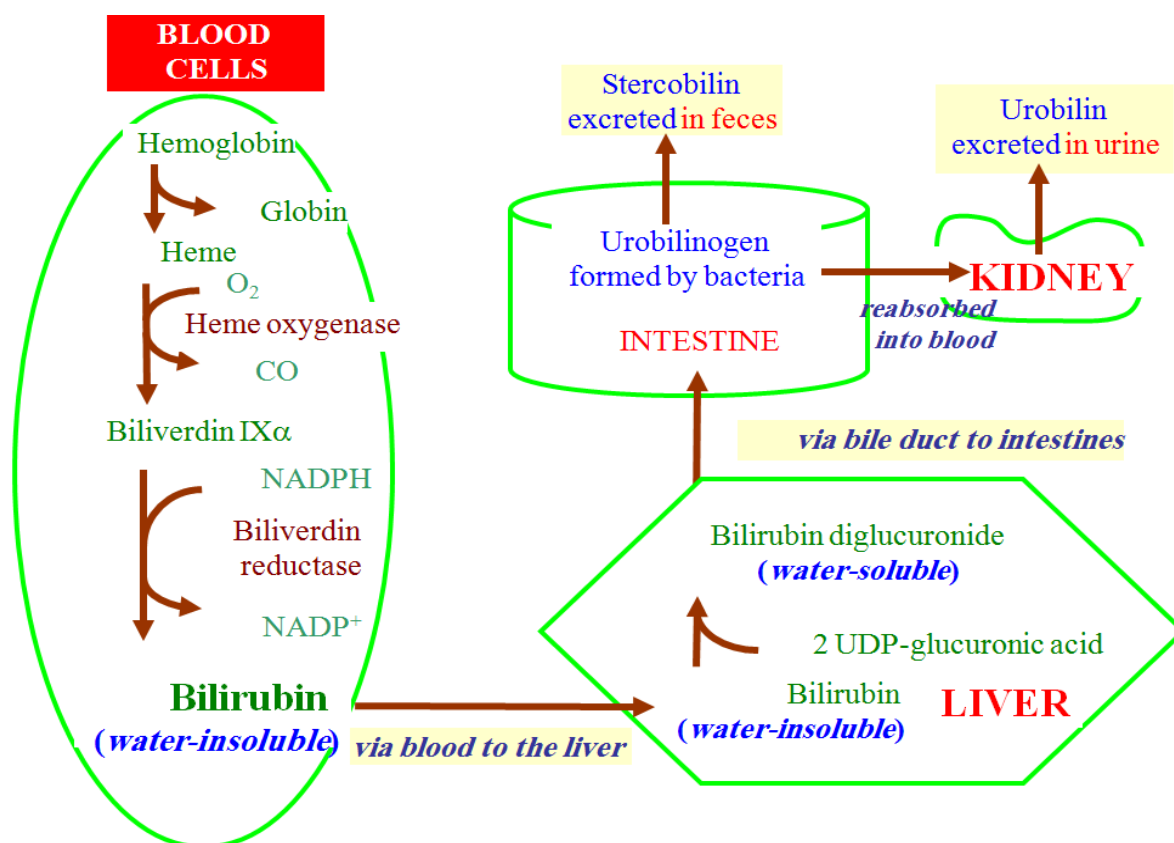


Figure (1) Bilirubin metabolism

Jaundice

Jaundice is the yellowish discoloration of the skin and sclera due to hyperbilirubinemia.

Bilirubin is a yellow pigment, its normal plasma level is 2-17 $\mu\text{mol/L}$. Normally, more than 95% of **bilirubin** in the **plasma** is **indirect (unconjugated)**. Jaundice becomes clinically apparent when the plasma bilirubin exceeds 50 $\mu\text{mol/L}$.

Jaundice may be classified into:

1- Pre-hepatic jaundice

The **production rate of bilirubin is increased**, exceeding the excretory capacity of the **liver**. Overproduction of bilirubin occurs in all forms of hemolytic anemia, less commonly, in conditions where there is much ineffective erythropoiesis (e.g. pernicious anemia). **Indirect (unconjugated) hyperbilirubinemia may occur in pre-hepatic jaundice**. **Bilirubin is not excreted in urine**. **Urinary urobilinogen concentration is increased**.

2- Hepatocellular jaundice

Hepatocellular damage due to viral hepatitis or toxins may interfere with the uptake of bilirubin, or with its conjugation or with secretion of conjugated bilirubin into bile. **Both indirect (unconjugated) and direct (conjugated) hyperbilirubinemia may occur in hepatocellular jaundice**. **Bilirubin and excess urobilinogen are found in urine**.

3- Post-hepatic (Obstructive) (Cholestatic) jaundice

It is due to impaction of **gallstones** in the common bile duct or **carcinoma** of the head of pancreas or of the biliary tree. **Direct (conjugated) hyperbilirubinemia may occur in obstructive Jaundice**. **Bilirubin is detected in urine**. It is characterized by **dark urine** and **pale stool**.

Class of Jaundice	Type of Bilirubin raised	Causes
Pre-hepatic or hemolytic	Unconjugated	Abnormal red cells; antibodies; drugs and toxins; thalassemia Hemoglobinopathies (Gilbert's, Crigler-Najjar syndrome)
Hepatic or Hepatocellular	Unconjugated and conjugated	Viral hepatitis, toxic hepatitis, intrahepatic cholestasis
Post-hepatic	Conjugated	Extrahepatic cholestasis; gallstones; tumors of the bile duct, carcinoma of pancreas

Congenital hyperbilirubinemias:

Include Gilbert's Disease (Syndrome), Crigler-Najjar Syndrome, Dubin –Johnson Syndrome and Rotor's Syndrome.

2. Albumin and prothrombin time

The measurement of plasma albumin and prothrombin time may be used to assess the hepatic synthetic function.

A. Albumin

Hypoalbuminaemia is such a common finding in many severe illnesses that it is a less specific indicator of impaired synthetic capacity than a prolonged prothrombin time. However, there are many other causes of a low plasma albumin concentration that are not due to hepatic disease such as loss into urine, loss from gut or (ascitis). Albumin has a long half-life of 20 days and levels fall slowly if no synthesis occur. Thus, serum albumin is usually normal in acute hepatitis. However, in chronic liver diseases such as cirrhosis, impaired synthesis may lead to low serum levels (i.e. a plasma albumin concentration below the lower reference limit may imply hepatic disease chronicity).

Serum **globulins** are usually **increased in cirrhosis** as a reflex to decrease albumin concentration.

B. Prothrombin time

The prothrombin time may be **prolonged** by **cholestasis**, fat-soluble **vitamin K cannot be absorbed normally** if fat absorption is impaired due to intestinal **bile salt deficiency**. The abnormality is then **corrected by parenteral administration of the vitamin K**. A prolonged prothrombin time may also result from **severe impairment of synthetic ability of clotting factors if the liver cell mass is greatly reduced**; in such cases it is **not corrected by parenteral administration of vitamin K**.

3. Liver enzymes

A. Aspartate transaminase (AST)

Aspartate transaminase is involved in amino acid metabolism. It has **widespread tissue distribution** including liver, red blood cells, skeletal and cardiac muscle. **No tissue specific isoenzymes**. Cytosolic and mitochondrial enzyme.

B. Alanine transaminase (ALT)

Alanine transaminase is involved in amino acid metabolism. It is **more liver specific**. Cytosolic enzyme only.

The **ALT is more specific for hepatic disease** because AST may be present also in skeletal muscle and other tissues. AST is more sensitive than ALT.

A rise in plasma aminotransferase activities is a sensitive indicator of damage to cytoplasmic and/or mitochondrial membranes. **Raised plasma transaminase concentrations are indicative of hepatocyte damage**, but do not necessarily reveal its mechanism. **In inflammatory or infective conditions, such as viral hepatitis**, the cytoplasmic membrane sustains the main damage; leakage of cytoplasmic contents causes a **relatively greater increase in plasma ALT than AST activities (acute hepatitis)**. **Infiltrative disorders** in which there is damage to both mitochondrial and

cytoplasmic membranes, there is a proportionally **greater increase** in plasma **AST** than **ALT** activity.

C. Alkaline phosphatase (ALP)

Alkaline phosphatase has **widespread tissue distribution** including liver, bone, placenta and GIT. The liver isoenzyme can be identified by electrophoresis. It is located on the outside of the cell membrane (canalicular side of liver cell). It is released into plasma in **cholestasis (obstructive)** because of **increased synthesis**.

D. Gamma-glutamyl transpeptidase (GGT)

Gamma-glutamyl transpeptidase is **more liver specific**. It is involved in the transport of amino acids across the liver cell plasma membrane. Serum level **increased** by **cholestasis (obstructive)** or **chronic ingestion of alcohol, barbiturates, phenytoin** and **other drugs which induce the enzyme**.

E. Lactate dehydrogenase (LDH)

Lactate dehydrogenase has **widespread tissue distribution** including liver, red blood cells, skeletal and cardiac muscle. Tissue specific isoenzymes can be separated by electrophoresis. The LD5 isoenzyme is found in liver and skeletal muscle only.

Cirrhosis

Cirrhosis is the **end result** of many inflammatory and metabolic diseases involving the liver, including **prolonged toxic damage**, usually due to **alcohol**. The fibrous scar tissue distorts the hepatic architecture, and regenerating nodules of hepatocytes disrupt the blood supply, sometimes increasing the pressure in the portal vein, causing portal hypertension. Blood may be shunted from the portal into the hepatic vein, bypassing the liver. **In the early stages there may be no abnormal biochemical findings.**

During phases of active cellular destruction, the plasma AST, and sometimes ALT, activities rise. **In advanced cases, the biochemical findings are mostly associated with a reduced functioning cell mass.** Portal hypertension and impaired lymphatic

drainage lead to fluid accumulation in the peritoneal cavity (**ascites**). This may be aggravated by **hypoalbuminaemia** (decrease synthesis of albumin), which may also cause peripheral **oedema**. In advanced cirrhosis, the findings of **hepatocellular failure** develop.

Neonatal Jaundice

- ✓ Physiologic jaundice (non-pathologic). There is **unconjugated** hyperbilirubinemia.
- ✓ Appears **after** 24 hours of birth. Normal full term babies may show jaundice between days 2 and 8 of life. Maximum intensity by 4th-5th day in term and 7th day in preterm.
- ✓ Plasma bilirubin level **rarely** exceeds 100 $\mu\text{mol/L}$.
- ✓ Transient, clinically not detectable after 14 days.
- ✓ Can disappear without any treatment.
- ✓ Red cell destruction (increased bilirubin load), together with immature hepatic processing of bilirubin because the **activity of bilirubin-UDP-glucuronyl transferase** is **low at birth** may cause a high plasma level of unconjugated bilirubin in the newborn infant

Non-physiologic jaundice (pathologic hyperbilirubinemia)

- There is **unconjugated or conjugated** hyperbilirubinemia **or both**.
- Appears **within** 24 hours of age (on the first day of life).
- Levels of bilirubin exceeding 100 $\mu\text{mol/L}$.
- Jaundice **persisting** after 14 days
- There are many **causes** including:
 - 1- Hemolytic disease: Rh and ABO incompatibility
 - 2- Infections: cytomegalovirus, malaria and bacterial infections
 - 3- G6PD deficiency
 - 4- Cirrhosis hepatitis and obstruction of common bile duct.

Bilirubin Neurotoxicity (kernicterus)

Unconjugated bilirubin is lipophilic and crosses the blood brain barrier (BBB). The BBB of infants is more permeable than adults. Unconjugated bilirubin has an affinity for the basal ganglia, hippocampus, cranial nerve nuclei. Unconjugated bilirubin interrupts metabolism in glial cells and causes apoptosis of neurons.

Treatment

- 1- **Purposes:** reduce level of serum bilirubin and prevent bilirubin toxicity
- 2- **Prevention of hyperbilirubinemia:** early feeds, adequate hydration
- 3- **Reduction of bilirubin levels:** phototherapy, exchange transfusion
- 4- **Drugs:** use of **phenobarbital** promote liver enzymes and protein synthesis