BILIRUBIN METABOLISM & JAUNDICE

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Brief description of Hemoglobin (Hb) structure

- Hemoglobin (Hb): Made up of 4-Subunits (Tetramer) held together by multiple non-covalent interactions;
- Each subunit consist of:
  - **Heme** (Ferro-Protoporphyrin),
  - **Globin** protein;
- **Heme**: Protoporphyrin IX and Ferrous ion ($\text{Fe}^2$);
- Globin protein folds around Heme group forming a protective Hydrophobic pocket;
- Heme is the site of Oxygen binding;
• There are different types of Hemoglobin, with different subunits:

• Foetal Hemoglobin (Hb F): $\alpha_2 \gamma_2$

Two types of Adult Hemoglobin (Hb A):

• Hb $A_1$ represented as: $\alpha_2 \beta_2$
  • It is the major (98%) form of Hb in adults;

• Hb $A_2$ represented as: $\alpha_2 \delta_2$
  • It is the minor (2%) form of Hb in adults;
What are the major sources of Heme in humans?

• RBC is major source of Heme in humans,
  • Life span of RBC is about 120 days,
• Other sources of Heme include:
  • Myoglobin (Mb): Stores Oxygen in muscle cells,
  • Cytochromes: present in some enzymes,
  • Catalase: an enzyme,
What normally happens to RBC after 120 days?

• RBC is destroyed mainly in Reticuloendothelial system (Extra-vascular system: Spleen and Liver);
• Daily turnover of Hb is about 6 g/day;
• Hb is broken down,
• Globin protein is hydrolyzed to amino acids,
• Protoporphyrin Ring in Heme is Hydrophobic, thus must be made soluble before it is excreted,
• Ferrous ion is removed and stored in Iron pool, for reuse,
• Protoporphyrin ring is metabolized to Bilirubin, mainly in Reticuloendothelial cells (spleen and bone marrow),
How is Heme metabolized to Bilirubin?

- Heme is degraded primarily in Reticuloendothelial cells by **Microsomal Enzyme system** that uses \( \text{O}_2 \) and \( \text{NADPH} \),
- **Heme Oxygenase** catalyzes break down of Heme to:
  - Biliverdin,
  - Ferric iron \((\text{Fe}^{3+})\), and
  - Carbon Monoxide (CO);
- Iron is stored for reuse,
- CO is excreted via lungs and can be measured in breath,
  - CO content of expired air is a direct measure of activity of Heme Oxygenase and rate of Heme metabolism,
• **Biliverdin** is reduced to **Unconjugated Bilirubin** in reaction catalyzed by **Biliverdin Reductase** that requires NADPH, (Fig. 1)

• Unconjugated Bilirubin is Hydrophobic;

• It is transported in blood tightly bound to albumin,

• Presence of endogenous and exogenous binding competitors, like certain drugs, decreases the binding affinity of albumin for bilirubin,

• Small fraction of unconjugated bilirubin in plasma is not bound to albumin,

• Free unconjugated bilirubin can cross cell membranes, including blood-brain barrier, leading to Neuro-toxicity,
Fig. 1: Metabolism of Heme to Bilirubin

- NADPH
- Heme
- Oxygenase
- Fe$^{3+}$ + NADP
- Carbon Monoxide
- Biliverdin
- Reductase
- NADPH
- Bilirubin
IMPORTANT TO NOTE

• 1.0g of Hb yields about 35.0mg of Bilirubin,
• In healthy adults: 250 to 350 mg Bilirubin is formed daily;
  • Derived mainly from Hb, ineffective Erythropoiesis, Mb and Cytochromes P\textsubscript{450};
• Bilirubin in excess of 25 mg in plasma can be bound only loosely to albumin, thus it can easily be displaced from albumin and diffuse into tissues,
• Antibiotics and other drugs compete with bilirubin for high-affinity binding sites on albumin,
• These compounds can displace bilirubin from albumin and therefore have significant clinical effects, especially in infants;
• Color of Biliverdin is Blue-Green,
• Color of Bilirubin is Yellow-Red,
• Change in color as Biliverdin is converted to Bilirubin is partly responsible for progressive changes in color of Hematoma, or Bruise, in which damaged tissue changes its color from:
  • Initial Dark Blue to Red-Yellow,
  • Finally to Yellow color before all the pigments are transported out of the affected tissue;
What are the stages of Bilirubin metabolism in liver?

- Metabolism of Bilirubin in liver is divided into three stages:
  - **Uptake** of Bilirubin by Hepatocytes,
  - **Conjugation** of Bilirubin in Smooth Endoplasmic Reticulum in Hepatocytes,
  - **Secretion** of Conjugated Bilirubin into Bile;

- Let us take a brief look at each stage;
- Fig. 2 is schematic diagram of Bilirubin Metabolism;
Briefly explain uptake of bilirubin by hepatocytes

• Bilirubin-albumin complex in plasma reaches the liver,
• Bilirubin enters Hepatocytes, and binds to Ligandin,
• Uptake of bilirubin into Hepatocytes increases with increasing Ligandin concentration,
• Ligandin concentration is low at birth, but increases rapidly over the first few weeks of life,
• Bilirubin is removed from albumin and taken up at the Sinusoidal surface of Hepatocytes by special transport system,
• Net uptake of bilirubin depends on removal of bilirubin by subsequent metabolic pathways in the liver,
How is Bilirubin conjugated in the liver?

- Conjugation: conversion of insoluble bilirubin to soluble bilirubin, which is readily soluble in bile,
  - It involves addition of 2 **Glucuronic Acids** to Bilirubin,
  - **Bilirubin Diglucuronide** is called **Conjugated Bilirubin**;
- Reaction is catalyzed by UDP-Glucuronyl Transferase located in the Smooth Endoplasmic Reticulum,
  
  **UDP-Glucuronyl Transferase reaction:**
  
  \[ 2 \text{UDP-Glucuronic Acid} + \text{Bilirubin} \rightarrow \text{Bilirubin Diglucuronide} \]
- Bilirubin excreted in bile is **Conjugated Bilirubin**,
How is Conjugated Bilirubin secreted into Bile?

• Secretion of conjugated bilirubin into bile occurs via Active Transport mechanism, which is rate-limiting for the process of hepatic bilirubin metabolism,

• Under normal physiological conditions, all Bilirubin secreted into bile is Conjugated,

• After Phototherapy, large amount of Unconjugated Bilirubin can be found in bile (Why?)

• Because Phototherapy converts unconjugated bilirubin to Lumirubin which is soluble in aqueous medium;
• De-conjugation of conjugated bilirubin by beta-Glucuronidase located in brush border, can occur in Proximal Small Intestine,

• Unconjugated bilirubin formed can be reabsorbed into circulation, increasing total plasma unconjugated bilirubin level,
  • Cycle of bilirubin Uptake, Conjugation, Excretion, De-conjugation, and Reabsorption is termed: **Enterohepatic Circulation of Bilirubin**;

• It occurs mainly in neonates;
How is Conjugated Bilirubin metabolized in Intestine?

- Bile containing conjugated bilirubin is released in GIT,
- Conjugated bilirubin may be De-conjugated by bacteria, resulting in Enterohepatic circulation of bilirubin,
- Fecal flora converts some conjugated bilirubin into Urobilinogens,
- Some urobilinogen is reabsorbed and re-excreted via liver to constitute Intra-hepatic Urobilinogen cycle,
- Some urobilinogen is excreted in the urine,
- Urobilinogen is excreted in feces and oxidized to Urobin,
- Darkening of feces in air is due to oxidation of residual Urobilinogen to Urobin,
Fig. 2: Metabolism of Bilirubin

1. Bilirubin + Albumin in Blood plasma
   → Bilirubin-Albunin

2. Bilirubin + Glucuronic Acid
   → Conjugated Bilirubin (in Liver)

3. Conjugated Bilirubin
   → Biliary system (Bile in Gall Bladder)

4. Conjugated Bilirubin
   → Urobilinogen

5. Urobilinogen
   → Urobilin (in Urine)

6. Urobilinogen
   → Action of Bacteria
   → Starchobilin

7. Starchobilin
   → Excreted in stool

Enterohepatic Circulation of Urobilinogen via Portal vein
What is Hyperbilirubinemia?

• Hyperbilirubinemia:
  • Accumulation of Bilirubin in blood,
  • Bilirubin level exceeds 1.0mg/dL (17.1 μmol/L),
List some causes of Hyperbilirubinemia

• Production of more bilirubin than normal liver can excrete,
• Failure of damaged liver to excrete bilirubin produced in normal amounts,
• Obstruction to excretory ducts of liver preventing excretion of bilirubin,
• Unconjugated Hyperbilirubinemia:
  • Accumulation of Unconjugated Bilirubin in blood;
• Conjugated Hyperbilirubinemia:
  • Accumulation of Conjugated Bilirubin in blood;
What are the 3 major causes of Hyperbilirubinemia?

• Hemolysis:
  • Increased Hb breakdown produces Bilirubin that overloads the conjugating mechanism in the liver,

• Failure of conjugating system in Hepatocytes,

• Obstruction in Biliary system,
How is Hyperbilirubinemia classified?

• Depending on the type of bilirubin (Conjugated bilirubin or Unconjugated bilirubin) present in the plasma, Hyperbilirubinemia may be classified as:

• **Retention Hyperbilirubinemia:**
  • Due to overproduction of bilirubin,
  • Accumulation of Unconjugated Bilirubin in blood;

• **Regurgitation Hyperbilirubinemia:**
  • Due to reflux of bilirubin into the blood stream because of biliary obstruction,
  • Accumulation of Conjugated and Unconjugated Bilirubin in blood;
What is Jaundice?

- **Jaundice (Icterus):** yellowish discoloration of Skin and Sclera due to deposit of Bilirubin,
  - When bilirubin in blood is about **2 – 2.5 mg/dl**, 
    (34.2 – 42.8µmol/L)
  - Hyperbilirubinemia can occur without jaundice, but jaundice cannot occur without Hyperbilirubinemia,
Simple classification of the causes of jaundice

• Causes of jaundice can be classified as follows:
  • Pre-hepatic jaundice (e.g., Hemolytic anemia),
  • Hepatic jaundice (e.g., Hepatitis),
  • Post-hepatic jaundice (Obstruction of common bile duct);
Other causes of Jaundice

Unconjugated Hyperbilirubinemia:

- Inherited disorders of Bilirubin metabolism leading to decreased clearance of bilirubin: Examples:
  - **Crigler-Najjar syndrome:**
    - Severe Unconjugated Hyperbilirubinemia due to Low activity of UDP-GT;
  - **Gilbert syndrome:**
    - Unconjugated Hyperbilirubinemia due to decreased expression of conjugating enzyme (UDP-GT)
IMPORTANT TO NOTE

• Encephalopathy due to Hyperbilirubinemia (Kernicterus) occurs in patients with unconjugated hyperbilirubinemia, as in Retention Hyperbilirubinemia;

• Conjugated bilirubin is soluble in aqueous medium, thus only Conjugated bilirubin can appear in urine,

• **Choluric Jaundice:** Choluria = presence of biliary pigment in urine, occurs in Regurgitation Hyperbilirubinemia (Why?)

• **Acholuric Jaundice:** No bile pigment in urine, occurs in Retention Hyperbilirubinemia, (Why?)
What lab tests are used for diagnosis of jaundice?

- **Liver Function Tests (LFT)** are the major lab tests for diagnosis of Jaundice:
  - Total Bilirubin,
  - Conjugated Bilirubin,
  - Transaminases (ALT & AST),
  - Alkaline Phosphatase (ALP),
  - Gamma Glutamyl Transpeptidase (GGTP),
  - Total Protein & Albumin,

- **Other tests may include:**
  - Urinary Urobilinogen and Bilirubin,
  - Inspection of color of Stool samples,
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