University of Papua New Guinea
School of Medicine and Health Sciences
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Discipline of Biochemistry and Molecular Biology
PBL SEMINAR

Hemolysis and Jaundice

What is Intravascular Hemolysis?

- Destruction of RBC (Hemolysis) normally occurs in Reticuloendothelial system (Extra-vascular compartment: Extravascular Hemolysis)
- In some diseases, Hemolysis of RBC occurs within the Vascular System (Intravascular compartment: Intravascular Hemolysis)
- During Intravascular Hemolysis Free Hb (Hemoglobin) and Heme are released in Plasma
    - Resulting in Free Hb and Heme being excreted through the Kidneys with substantial loss of Iron
  - Loss of Iron is prevented by Specific Plasma Proteins:
    - Transferrin and Haptoglobins are involved in scavenging mechanisms
      - Transferrin is the protein that binds and transports Iron in plasma and thus permits Reutilization of Iron
      - Haptoglobins are a group of proteins, all of which are β_2-Globulins produced in the Liver

What happens to Free Hb during Intravascular Hemolysis?

- Sequence of events that occurs when Free Hb appears in plasma **(Fig. 1):**
    - Hb is Oxygenated in Pulmonary Capillaries,
    - OxyHb dissociates into αβ-OxyHb Dimers
    - αβ-OxyHb Dimers are then bound to circulating plasma Haptoglobins
        - Haptoglobins have High Affinity for αβ-OxyHb Dimers
    - One molecule of Haptoglobin binds two αβ-OxyHb Dimers
    - DeoxyHb does not dissociate into Dimers under normal physiological settings, thus it is not bound by Haptoglobins
    - Complex formed when Haptoglobin interacts with αβ-OxyHb Dimers is usually too large to be filtered through Renal Glomerulus
    - During Intravascular Hemolysis Free Hb, appears in Renal Tubules and in Urine (causing **Black-Water Fever**) only when the binding capacity of circulating Haptoglobin molecule has been exceeded

What are the functions of Haptoglobin?

- Prevent loss of Free Hb via the Kidneys
- Haptoglobin binds and transports αβ-OxyHb Dimers to Lymphoreticular system for catabolism
- Heme in Free Hb is relatively resistant to the action of Heme Oxygenase
    - Heme Oxygenase easily catalyzes breakdown of Heme in the Haptoglobin-αβ-OxyHb Complex

How significant is plasma Haptoglobin as a diagnostic tool?

- Measurement of Plasma Haptoglobin level is used clinically to indicate severity of Intravascular Hemolysis
  - **Patients with significant Intravascular Hemolysis have low levels of Haptoglobin because of removal of Haptoglobin-αβ-OxyHb complexes by Reticuloendothelial system**
  - Plasma Haptoglobin level falls rapidly when Intravascular Hemolysis is increased (e.g., Hemolytic Anemia); Free Haptoglobin may then be undetectable in Plasma
- Haptoglobin levels can also be low in Severe Extra-vascular Hemolysis, when large amount of Hb in the Reticuloendothelial System leads to transfer of Free Hb into plasma
- Decreased Plasma Haptoglobin level may occur in Liver disease
- Plasma Haptoglobin level increases in:
  - Acute Infections, Trauma, Nephrotic syndrome (**Why?)**
    - Because Haptoglobin is one of the Acute-Phase Reactants

<u>HEMOLYSIS AND G-6-P D DEFICIENCY</u>:

What reaction does Glucose-6-Phosphate Dehydrogenase catalyze?

- Glucose-6-Phosphate Dehydrogenase (G-6-P D) catalyzes the first reaction in the **HMP-shunt**
- NADPH is produced in the reaction catalyzed by G-6-P D
- HMP shunt that occurs in the RBC is important for maintaining Integrity of RBC membrane (**Why?**)
  - Because the NADPH produced is used to protect the integrity of RBC membrane by maintaining normal cellular level of **Reduced Glutathione (GSH)**

How do GSH and G-6-P D interact to protect RBC membrane from damage by Oxidants?

- Oxidants can damage RBC membrane causing Hemolysis
- GSH is a reducing agent that removes Oxidants in RBC
- For example:
  - **GSH** interacts with Oxidants in reaction catalyzed by **Glutathione Peroxidase** (Selenium is required)
  - In the process **GSH** is oxidized to **Oxidized Glutathione (GSSG**)

$$\mathbf{2GSH + H_2O_2 ========\rightarrow GSSG + 2H_2O}$$

- **GSSG** formed must be converted back to **GSH**, in a reaction catalyzed by **Glutathione Reductase** that utilizes **NADPH**

$$\mathbf{GSSG + NADPH + H^+ ======\rightarrow 2GSH + NADP^+}$$

- Major source of **NADPH** is the **G-6-P D** reaction in HMP shunt
  - HMP shunt is the only means of producing NADPH in mature RBC
- Decreased level of GSH in RBC results in accumulation of Oxidants, causing impairment of essential metabolic processes and Hemolysis

What are some of the consequences of G-6-P D deficiency?

- Mature RBC is very sensitive to Oxidative damage if the function of HMP shunt is Impaired (e.g., by G-6-P D deficiency)
- Oxidants (e.g., Anti-malarial drug Primaquine and other drugs) can interact with GSH to produce high amount of GSSG, which must be converted to GSH using NADPH from HMP shunt
- Mature RBC of individuals who are deficiency in G-6-P D cannot generate sufficient NADPH to convert GSSG to GSH
    - Resulting in accumulation of GSSG, this impairs the ability of RBC to dispose of Oxidants and Free Radicals (Reactive Oxygen Species)

- Accumulation of Oxidants and Free Radicals cause Oxidation of critical –SH groups in proteins and Peroxidation of Lipids in RBC membrane, causing Hemolysis **(Figs. 2 & 3)**
- Administration of Drugs or Chemical agents capable of generating Oxidants to G-6-P D deficient individuals can cause rapid fall in GSH level in mature RBC, leading to Intravascular Hemolysis
- Effect of G-6-P D deficiency is greatest in Older RBC, because of their inability to synthesize Protein and produce more G-6-P D
    - Mature RBC cannot synthesize protein and is devoid of Nucleus
- Hemolysis is higher in Older RBC, which explains the high percentage of circulating Young RBC usually found in blood of patients with Intravascular Hemolysis
- Hemolysis may be accompanied by unconjugated bilirubinemia leading to jaundice

HYPERBILIRUBINEMIA AND JAUNDICE

What is Hyperbilirubinemia?

- Hyperbilirubinemia:
    - Accumulation of Bilirubin in blood, when level of Bilirubin exceeds 1.0 mg/dL (17.1 μmol/L),

What are the different types of Hyperbilirubinemia?

- **Pre-hepatic Hyperbilirubinemia**:
    - Due to over-production of bilirubin causing increased level of unconjugated bilirubin in plasma:
    - Occurs in:
        - Hemolytic anemia
        - Hemolytic disease of the new-born, due to rhesus incompatibility
        - Ineffective Erythropoiesis (e.g., Pernicious Anemia)
        - Bleeding into tissues (Trauma)
        - Rhabdomyolysis

- **Hepatocellular Hyperbilirubinemia**:
    - May be due to:
        - Hepatocellular damage caused by:
            - Infective agents, Drugs or Toxins
            - Cirrhosis is usually a late complication
        - Low activity or Failure of the conjugating mechanism: UDP-Glucuronyl-Transferase within the Hepatocytes,

- **Cholestatic Hyperbilirubinemia**:
  - Cholestasis may be **Intra-hepatic or Extra-hepatic**
    - Both causes Conjugated Hyperbilirubinemia and Bilirubinuria

- **Intra-hepatic Cholestasis** commonly due to:
    - Acute Hepatocellular damage (e.g., Infectious Hepatitis)
    - Primary Biliary Cirrhosis
    - Drugs

- **Extra-hepatic Cholestasis** is most often due to:
    - Gallstones
    - Carcinoma of Head of Pancreas
    - Carcinoma of Biliary Tree
    - Bile duct compression from other courses

How is Hyperbilirubinemia related to Jaundice?

- Jaundice (French: jaune: Yellow) is due to Hyperbilirubinemia
  - Jaundice is seen clinically when level of Bilirubin in blood exceeds **2.5 mg/dL**
  - Bilirubin diffuses into some Tissues, such as the Sclera, which then become yellow (**Jaundice or Icterus**)
  - Yellow discoloration of the eyes in Jaundice patients is due to affinity of the protein Elastin (in Sclera) for Bilirubin
  - Elastin in Sclera does not bind Carotene, thus hyper-carotenemia does not cause yellow discoloration of the eyes

What are the two types of Hyperbilirubinemia?

- Hyperbilirubinemia can be separated based on the type of Bilirubin (Conjugated Bilirubin or Unconjugated Bilirubin) present in Plasma,
    - **Retention Hyperbilirubinemia**: due to overproduction of bilirubin,
    - **Regurgitation Hyperbilirubinemia**: due to reflux of bilirubin into the blood stream because of biliary obstruction
- Unconjugated bilirubin is Hydrophobic, thus it can cross the Blood-Brain Barrier and enter the Central Nervous System
- Encephalopathy due to Hyperbilirubinemia (Kernicterus) can occur only in connection with Unconjugated Hyperbilirubinemia – as in Retention Hyperbilirubinemia
- Conjugated Bilirubin is Hydrophilic (i.e., soluble in water), thus conjugated bilirubin can appear in Urine
- **Choluric Jaundice** (Choluria = presence of biliary derivatives in urine) occurs only in Regurgitation Hyperbilirubinemia (high conjugated bilirubin in plasma)
- **Acholuric Jaundice** occurs in Retention Hyperbilirubinemia (high Unconjugated bilirubin in plasma)

How the causes of Jaundice be classified?

- Causes of Jaundice can simply be classified as follows:
  - Pre-hepatic Jaundice (e.g., Hemolytic anemia),
  - Hepatic Jaundice (e.g., Hepatitis),
  - Post-hepatic Jaundice (e.g., Obstruction of the common bile duct)

What laboratory tests can identify the different classes of Jaundice?

- Liver Function Tests is recommended
- Other tests: (See **Table below**)
  - Plasma Total Bilirubin and Conjugated Bilirubin,
  - Urinary Urobilinogen
  - Urinary Bilirubin,
  - Inspection of Stool Samples

Laboratory results for Healthy patient and patients with 3 different causes of Jaundice

Patients	Serum Bilirubin (mg/dl)	Urine Bilirubin	Urine Urobilinogen (mg/24h)	Fecal Urobilinogen (mg/24h)
Normal	Direct: 0.1 – 0.4 Indirect: 0.2 – 0.7	Absent	0 – 4	40 – 280
Hemolytic Anemia	Elevation of Indirect	Absent	Increased	Increased
Hepatitis	Elevations of Direct & Indirect	Present	Decreased	Decreased
Obstructive Jaundice	Elevation of direct	Present	Absent	Trace to absent

TAKE NOTE:
Direct Bilirubin: Conjugated Bilirubin
Indirect Bilirubin: Unconjugated Bilirubin