

University of Papua New Guinea
 School of Medicine and Health Sciences
 Division of Basic Medical Sciences
 Discipline of Biochemistry and Molecular Biology
 PBL SEMINAR
HEMOLYSIS AND JAUNDICE: An overview

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:
 - Hb is Oxygenated in Pulmonary Capillaries,
 - OxyHb dissociates into $\alpha\beta$ -OxyHb Dimers
 - $\alpha\beta$ -OxyHb Dimers are then bound to circulating plasma Haptoglobins
 - Haptoglobins have High Affinity for $\alpha\beta$ -OxyHb Dimers
 - One molecule of Haptoglobin binds two $\alpha\beta$ -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 $\alpha\beta$ -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 $\alpha\beta$ -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- $\alpha\beta$ -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- $\alpha\beta$ -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

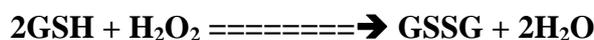
HEMOLYSIS AND G-6-P D DEFICIENCY:

What reaction does Glucose-6-Phosphate Dehydrogenase catalyze?

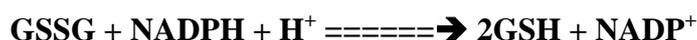
- ❑ Glucose-6-Phosphate Dehydrogenase (G-6-P D) catalyzes the first reaction in the **HMP-shunt**
- ❑ NADPH is produced in the reaction catalyzed by G-6-P D
- ❑ HMP shunt that occurs in the RBC is important for maintaining Integrity of RBC membrane (**Why?**)
 - Because the NADPH produced is used to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



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



- ❑ 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
- ❑ 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 $\mu\text{mol/L}$),

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