HEMOLYSIS & JAUNDICE: An Overview

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What do you understand by 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 Hemoglobin and Heme are released in Plasma;

- Free Hb and Heme in plasma can result in their excretion via the Kidneys with substantial loss of Iron
- Loss of Iron is prevented by Specific Plasma Proteins:
  - **Transferrin,** and
  - **Haptoglobins (Hp)**
    - Both are involved in scavenging mechanisms
- **Transferrin:** binds and transports Iron in plasma and thus permits Reutilization of Iron;
- **Haptoglobins (Hp):** β_2-Globulins produced in the Liver;

What happens to Free Hb during Intravascular Hemolysis?

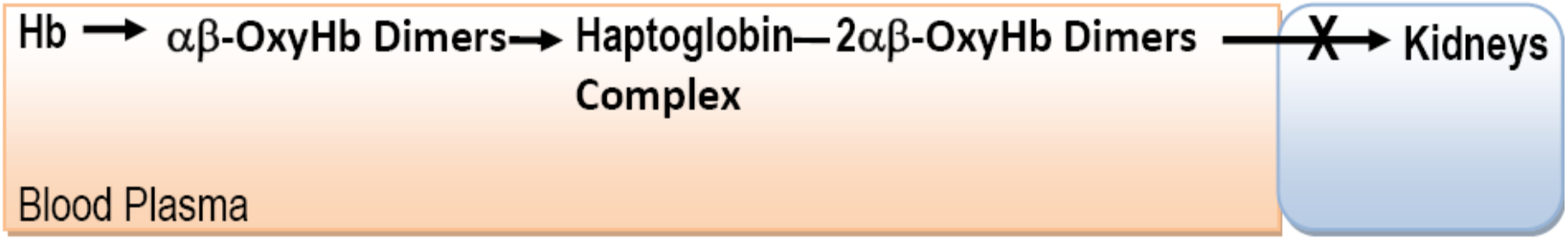
- Sequence of events that occurs when Free Hb appears in plasma **(Fig. 1):**
  - Hb is Oxygenated in Pulmonary Capillaries,
  - OxyHb dissociates into **αβ-OxyHb Dimers,**
  - αβ-OxyHb Dimers are bound by circulating plasma Haptoglobins,
    - Haptoglobins have High Affinity for αβ-OxyHb Dimers;
- One 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 $\alpha\beta$-OxyHb Dimers is too large to be filtered via the Glomerular Membrane;
- During Intravascular Hemolysis:
  - Free Hb, appears in Renal Tubules and in Urine, causing **Black-Water Fever**,
  - This occurs when binding capacity of circulating Haptoglobin molecules have been exceeded;

Figs. 1 & 2

Schematic diagram of Free Hb filtration via glomerular membrane into urine

Schematic diagram of Hb-Haptoglobin complex that cannot be filtered via the glomerular membrane because of very large size of the complex



What are the functions of Haptoglobin?

- **Haptoglobins (Hp)**:
  - Prevent loss of Free Hb via the Kidneys,
  - Binds and transports $\alpha\beta$-OxyHb Dimers to liver and 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 during severe Intravascular Hemolysis (Hemolytic Anemia)
  - Level of Haptoglobin in Plasma will be very low;

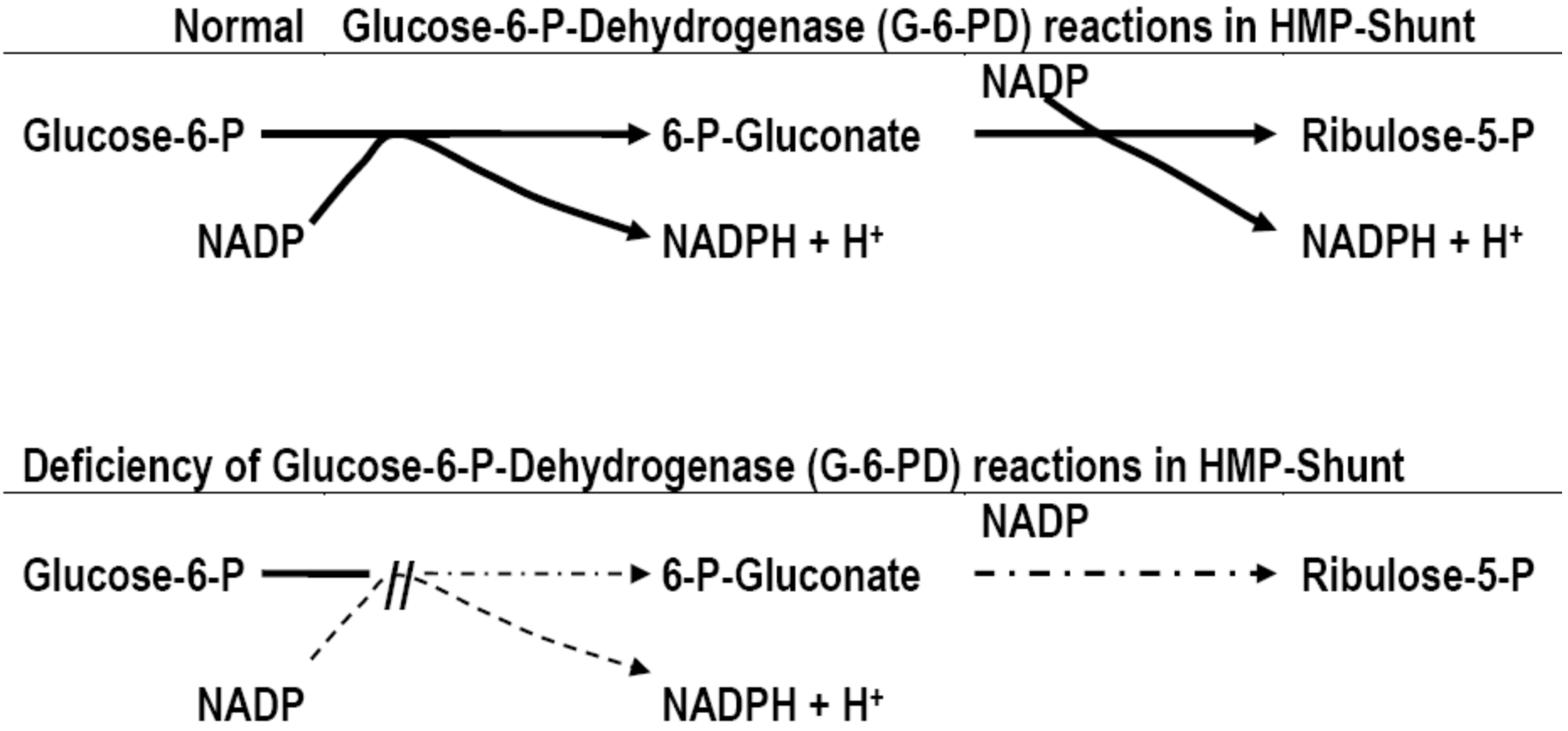
- Haptoglobin levels in plasma can also be low during **Severe Extra-vascular Hemolysis**, when large amount of Hb in Reticuloendothelial System leads to release of Free Hemoglobin in plasma,
- Decreased Plasma Haptoglobin level may occur in Liver disease,
- Plasma Haptoglobin level increases in:
    - 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 catalyzes?

- Glucose-6-Phosphate Dehydrogenase (**G-6-PD**) catalyzes the first reaction in **HMP-shunt (Fig. 3)**
- **NADPH** is produced in reaction catalyzed by G-6-PD,
- HMP shunt in RBC is important for maintaining the Integrity of RBC membrane (**Why?**)
  - Because the NADPH produced is used to protect the integrity of RBC membrane by maintaining normal cellular levels of **Reduced Glutathione (GSH);**
- **GSH: Reduced Glutathione**
- **GSSG: Oxidized Glutathione**

Fig. 3: Glucose-6-Phosphate Dehydrogenase (G-6-PD) reaction



How do GSH and G-6-PD 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,
- Process is as follows:
  - **GSH** interacts with Oxidants to form **GSSG,**
    - Reaction is catalyzed by **Glutathione Peroxidase** (**Selenium** is required),
  - **GSSG** then reacts with **NADPH + H^+** to form **GSH**,
    - Reaction is catalyzed by **Glutathione Reductase** that utilizes **NADPH**

Glutathione Peroxidase reaction (Selenium is required)

$$2GSH + 2H_2O_2 \xrightarrow{\quad\quad} GSSG + 2H_2O$$

Glutathione Reductase reaction

$$GSSG + NADPH + H^+ \xrightarrow{\quad\quad} 2GSH + NADP^+$$

- Major source of **NADPH** is the **G-6-PD** reaction in HMP shunt,
- HMP shunt is the major pathway for producing NADPH in mature RBC,
- **Decreased in the level of GSH in RBC results in accumulation of Oxidants, causing impairment of essential metabolic processes and Hemolysis;**

What are some consequences of G-6-PD deficiency?

- **Mature RBC** is sensitive to **Oxidative damage** if function of HMP shunt is Impaired (e.g. G-6-PD deficiency);
- Oxidants (e.g. Primaquine and other Anti-malarial 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 **deficiency in G-6-PD cannot** generate sufficient **NADPH** to convert **GSSG** to **GSH;**
  - Resulting in accumulation of GSSG in the RBG;
  - This impairs the ability of RBC to dispose of Oxidants and Free Radicals (Reactive Oxygen Species);

- Accumulation of Oxidants and Free Radicals causes Oxidation of critical –SH groups in proteins and Peroxidation of Lipids in RBC membrane, causing Hemolysis; **(Figs. 3 & 4)**
- Drugs or Chemicals capable of generating Oxidants should not be given to individuals with G-6-PD deficient; (**WHY?**)
  - Because they can cause rapid fall in GSH level in mature RBC, leading to Intravascular Hemolysis;

- Effect of G-6-PD deficiency is greatest in Older RBC, because of their inability to synthesize Proteins and produce more G-6-PD,
  - Mature RBC cannot synthesize proteins because they do not contain Nucleus;
- Hemolysis is higher in Older RBC,
  - This explains the high percentage of circulating Young RBC in blood of patients with Intravascular Hemolysis
- Hemolysis may be accompanied by unconjugated hyperbilirubinemia leading to jaundice;

Fig. 3: Glucose-6-Phosphate Dehydrogenase (G-6-PD) reaction

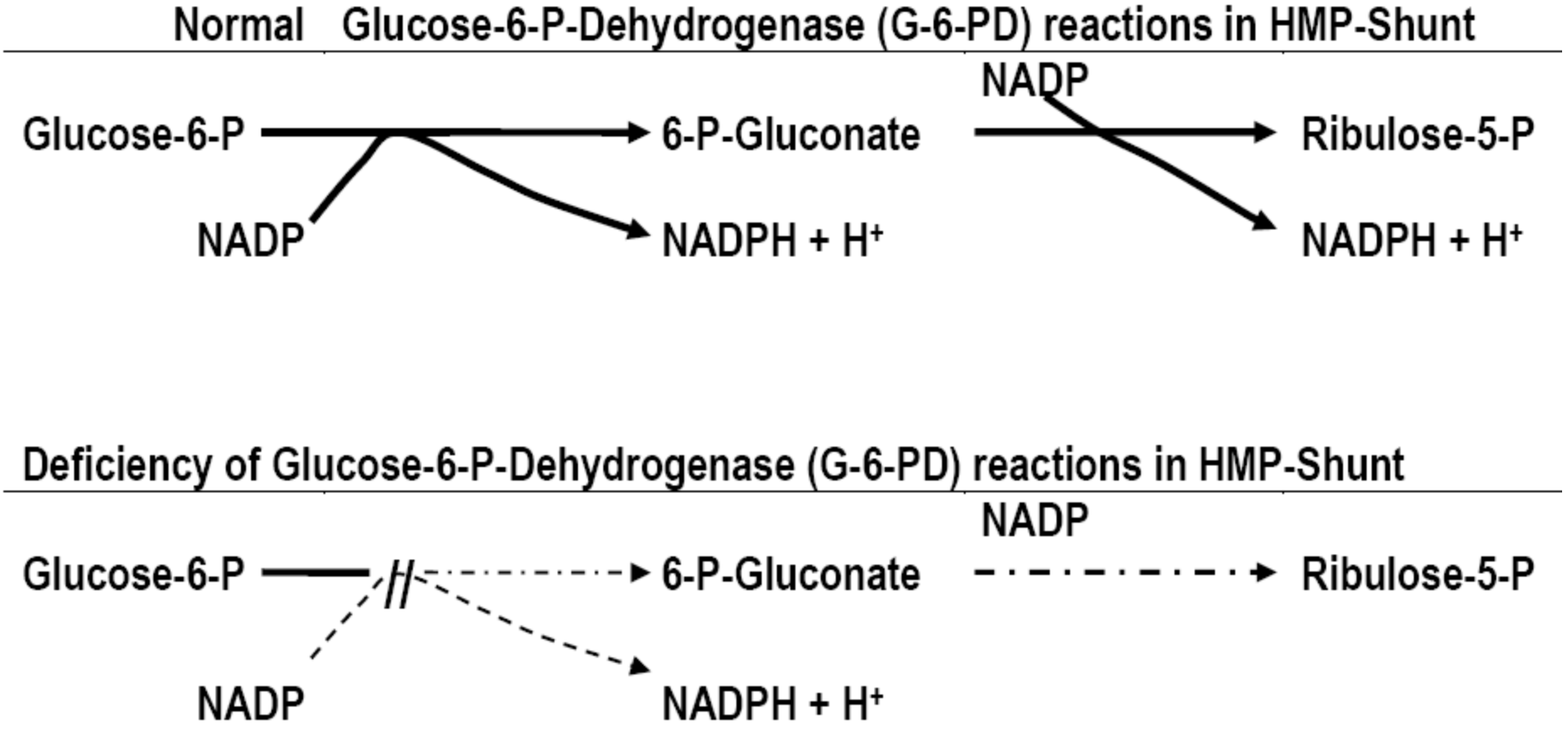


Fig. 4: Cellular protection of damage by Primaquine and other oxidants

Glutathione Peroxidase reaction (Selenium is required)

$$2\ GSH + Primaquine \longrightarrow GSSG + 2H_2O$$

$$2\ GSH + Protein\text{-}S\text{-}S\text{-}Protein \longrightarrow GSSG + 2Protein\text{-}SH$$

Glutathione Reductase reaction

$$GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+$$

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:
- May occur 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 /Failure of Conjugating mechanism
    - UDP-Glucuronyl-Transferase within Hepatocytes,

- **Cholestatic Hyperbilirubinemia:**
  - may be Intra-hepatic or Extra-hepatic
  - Causes Conjugated Hyperbilirubinemia and Bilirubinuria
- **Intra-hepatic Cholestasis** commonly due to:
  - Acute Hepatocellular damage (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**: due to Hyperbilirubinemia,
  - It occurs when plasma Bilirubin exceeds **2.5mg/dL,**
  - Bilirubin diffuses into skin and Sclera, which then become yellow (**Jaundice or Icterus**),
  - Yellow discoloration of eyes in Jaundice patients is due to affinity of protein **Elastin** (in **Sclera**) for Bilirubin,
  - Hypercarotenemia (high beta-carotene level in blood) does not cause yellow discoloration of the eyes because Elastin does not bind beta-carotene,

What are the two types of Hyperbilirubinemia?

- **Hyperbilirubinemia:** separated based on type of Bilirubin (**Conjugated Bilirubin** or **Unconjugated Bilirubin**) present in Plasma:
- **Retention Hyperbilirubinemia**: overproduction of bilirubin, leading to accumulation in blood;
- **Regurgitation Hyperbilirubinemia**: hepatic reflux of bilirubin into blood caused by biliary obstruction,

- **Unconjugated bilirubin is Hydrophobic, thus it can cross Blood-Brain Barrier and enter Central Nervous System,**

- **Encephalopathy** due to Hyperbilirubinemia (Kernicterus) can occur only in Unconjugated Hyperbilirubinemia – as in **Retention Hyperbilirubinemia**;
- Conjugated Bilirubin is Hydrophilic, thus it can appear in Urine;
- **Choluric Jaundice** (Choluria: biliary pigments in urine):
  - Occurs in Regurgitation Hyperbilirubinemia, **WHY??**
  - Because of high Conjugated Bilirubin in blood;
- **Acholuric Jaundice** (Acholuria: no biliary pigments in urine)
  - Occurs in Retention Hyperbilirubinemia, **WHY??**
  - Because of high Unconjugated bilirubin in blood,

How are the causes of Jaundice classified?

- Causes of Jaundice are simply classified as:
  - 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 are used for diagnosis of different classes of Jaundice?

- Several lab tests including the following:
  - Liver Function Tests (LFT),
  - Plasma Total Bilirubin and Conjugated Bilirubin,
  - Urinary Urobilinogen,
  - Urinary Bilirubin,
  - Inspection of Stool Samples
- Examples: **Table 1**

Table 1: Laboratory results for Healthy patient and patients with 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

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