NEONATAL Hb, O$_2$-TRANSPORT & JAUNDICE: OVERVIEW

UNIVERSITY OF PNG
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DISCIPLINE OF BIOCHEMISTRY & MOLECULAR BIOLOGY
PBL MBBS IV SEMINAR

VJ Temple
Brief description of the structure of Haemoglobin (Hb)

- Hb consist of 4-Subunits (Tetramer) held together by multiple non-covalent interactions;
- Each subunit consist of: Haem (Ferro-Protoporphyrin) and Globin protein;
- Haem: Protoporphyrin IX and Ferrous ion (Fe$^2+$);
- Globin protein folds around Haem forming a protective Hydrophobic pocket;
- Haem is the site of Oxygen binding;
- Different types of Hb (Hb F, Hb A) in humans;
- Primary structure of Globin is difference in Hb types;
- Subunits in Hb are products of different genes;
How is expression of Genes related to Types of Hb (structure of Hb)?

• Time of expression of normal genes for particular Hb type depends on the need for Oxygen transport at the stage of development (Fig. 1)

Embryogenesis:

• Initial Hb type is Tetramer ($\zeta_2 \epsilon_2$):
  • Two Zeta ($\zeta$) subunits, (which are evolutionally similar to $\alpha$ subunits,) and
  • Two Epsilon ($\epsilon$) subunits;
Through First Six months of development:

- Zeta (ζ) subunits are replaced by **Alpha (α)** subunits,
- Epsilon (ε) subunits are replaced by **Gamma (γ)** subunits
- Forming $\alpha_2 \gamma_2$ Foetal Hb (Hb F);

- Through later embryonic development and shortly before birth **γ-chain** synthesis diminishes and **β-chain** synthesis is initiated;
- Thus, **Beta (β) subunits** replaced **Gamma (γ) subunits**;
- Forming $\alpha_2 \beta_2$ Adult Hb (Hb A);
- Compared to Adult Hb ($\alpha_2 \beta_2$) Foetal Hb (Hb F) is the major Hb in Neonates;
• Hb F has two γ-chains in place of two β-chains in Hb A;
• By age of between Six and Seven months over 90% of Infant’s haemoglobin is Hb A;

Two types of Hb A:
• Hb A₁ (α₂ β₂) is the major (98%) form of Hb A in adults;
• Hb A₂ (α₂ δ₂) is a minor (2%) form of Hb A in adults;
Fig. 1: Diagram of expression of genes for different Globin chains in Hb during early development (Davidson & Sittman: Biochemistry 3rd Ed)
How is the structure of Hb related to functions (Structure – Function relationships)?

- Mechanism of Cooperative binding of O₂ to Hb, allosteric effects of H⁺, CO₂ and 2,3-BPG on Hb emphasizes the role structure plays in the function of Hb (Figs 2a, 2b, Fig 3)
- Hb F is adapted to the environment of the Foetus that gets O₂ from Maternal blood;
- Foetus must pick up O₂ at the Low pO₂ of the Placenta,
- Structure of Hb F is different from Maternal Hb that releases the O₂ in the placenta;
- Affinity of Hb F for O₂ is Higher than that of Hb A for O₂
- Higher affinity of Hb F for O₂ is because γ-subunits does not bind 2,3-BPG well;
Figs 2a, 2b: Action of allosteric effectors on Oxygen binding curve of HbA,
Fig. 3: Diagram of binding of 2,3-BisPhosphoglycerate (2,3-BPG)
• $O_2$-binding curve of Hb F is shifted to Left of Hb $A_1$ (Fig. 4)
• 15 to 20% of Hb F is acetylated at N-terminals (Hb F$_1$);
• Hb F$_1$ does not bind 2,3-BPG; thus 2,3-BPG does not affect its affinity for $O_2$
• Postnatal change from Hb F to Hb A, and increase of RBC levels of 2,3-BPG that peaks 3 months after birth, results in gradual shift to the Right of the Oxygen-binding curve in Infants;
• Resulting in greater delivery of Oxygen to Tissues at this stage than at birth, in spite of a 30% decrease in total Hb level in infants;
Fig. 4: Comparison of Oxygen-binding curves for Hb F and Hb A
Outline the metabolism of bilirubin.

• Bilirubin produced in Reticuloendothelial system during Haem catabolism;
• Heme Oxygenase catalyses Heme to Biliverdin, Fe$^{2+}$, CO;
• CO excreted via Lungs; can be measured in breath to quantify bilirubin production;
• Biliverdin reduced to Unconjugated Bilirubin,
• Unconjugated bilirubin transported in plasma bound mainly to Albumin,
• Binding of bilirubin to albumin increases Postnatal with age and is reduced in sick infants;
• Presence in blood of endogenous and exogenous (certain drugs) binding competitors decreases binding affinity of Albumin for bilirubin;
• Small fraction of unconjugated bilirubin in plasma not bound to albumin can cross cell membrane, BBB, leading to Neurotoxicity;

• Bilirubin-Albumin complex is transported into Hepatocytes and binds to Ligandin,

• Ligandin levels are low at birth, but increase rapidly over the first few weeks of life,

• Pharmacologic agents (e.g., Phenobarbital) increase concentration of Ligandin,

• **UDP-Glucuronyl-Transferase** (UDP-GT) catalyses Conjugation of Bilirubin in Hepatocytes,
• Conjugated Bilirubin is hydrophilic, thus excreted in bile,

• Activity of UDP-GT is low at birth, but increases to adult values by age 4 - 8 weeks;

• Certain drugs (Phenobarbital, Dexamethasone, Clofibrate) can increase UDP-GT activity;

• Conjugated Bilirubin in bile released in Intestine,

• Some Conjugated Bilirubin are metabolised by Microbes in Colon to form colourless compounds (Mesobilinogens or Urobilinogens);
• De-conjugation occurs in Proximal Small Intestine by **Beta-Glucuronidase** in Brush Border,
• Unconjugated bilirubin formed is reabsorbed into circulation, increasing Bilirubin level in plasma,
• Cycle of Uptake, Conjugation, Excretion, De-conjugation and Reabsorption is termed **Enterohepatic Circulation of Bilirubin**,  
  • It occurs mainly in Neonates;
How is bilirubin cleared from Foetal blood? (Prenatal Clearing of bilirubin)

• Unbound Foetal bilirubin continuously crosses Placenta into Mother blood because of greater albumin-binding capacity of Maternal plasma;
• Bilirubin from Foetus is Conjugated in Maternal Liver by UDP-Glucuronyl-Transferase (UDP-GT);
• UDP-GT in healthy mothers is in great excess, thus high bilirubin level of major haemolytic events in foetus do not exceed the capacity of maternal UDPGT to conjugate bilirubin;
• Conjugated bilirubin formed in maternal blood is secreted by active transport into bile canaliculi, concentrated in Gallbladder and excreted in the intestinal tract;

• De-conjugation and re-sorption of bilirubin is minimal in healthy adults;

• Intestinal bacteria metabolises the conjugated bilirubin to Stercobilinogen and Urobilinogen that are excreted in maternal stool and urine;
How is bilirubin metabolised in Neonate? (Neonatal Metabolism of Bilirubin)

- Bilirubin Conjugation is limited in Foetus and Neonate, because of Immaturity of Conjugating enzyme (UDPGT);
- At birth: UDPGT in liver is 0.1% to 1.0% that of adult,
- UDPGT increases over time but does not reach adult levels until about 6 to 14 weeks after birth,
- Daily bilirubin excretion in Neonate is disproportionately large
- Thus, bilirubin accumulates in blood of all Neonates,
- A twofold increase in neonatal bilirubin production occurs because of:
  - Higher circulating erythrocyte volume,
  - Change in the life span of the erythrocytes
• Intestinal Beta-Glucuronidase is high in Neonates, resulting in increased de-conjugation of conjugated bilirubin,

• Thus, greater re-sorption of unconjugated bilirubin through Enterohepatic circulation of bilirubin;

• Certain factors in Breast-milk of some mothers contribute to increased Enterohepatic circulation of bilirubin (breast-milk jaundice);
• It is significant for breast-fed babies, who receive additional Beta-Glucuronidase in breast milk;
• Infants lack Intestinal bacterial flora, thus very little conjugated bilirubin is converted to Stercobilinogen and Urobilinogen,
• Thus, both conjugated and unconjugated bilirubin are excreted as Golden-yellow pigment characteristic of the stools of Neonate;
HYPERBILIRUBINEMIA IN NEONATES

• Some health term Neonates develop hyper-bilirubinemia to a greater or lesser degree, in the first week of life,

• This is made due to several reasons:
  • Increased production of bilirubin (accelerated red blood cell breakdown),
  • Decreased removal of bilirubin (transient liver enzyme insufficiency),
  • Increased Enterohepatic circulation of bilirubin,
  • Lower Albumin concentration in Plasma,
Factors that affect the capacity of Albumin to bind and transport bilirubin include:

- Acid – Base disturbances
- Presence of drugs (e.g., Sulphonamides)

Hyperbilirubinemia in Neonates is Primarily due to Immaturity of the Conjugating enzyme (UDPGT);

Severe unconjugated hyperbilirubinemia in neonates can cause *Kernicterus* (bilirubin encephalopathy):

- Staining of Basal Ganglia by unconjugated bilirubin,
- It involves diffuse neuronal damage causing severe neurologic consequences,
Jaundice in Neonates can be Physiologic or Non-physiologic (Pathologic) according to:

- Post-delivery timing of onset,
- Clinical course,
- Resolution,
- Rate of bilirubin increases,
- Total serum bilirubin levels.
Neonatal Hyperbilirubinemia leading to Jaundice

• **Hyperbilirubinemia** occurs in nearly all newborns and can be classified in several categories:
  • Physiologic jaundice of the newborn,
  • Non-Physiologic or Pathologic jaundice,
  • Breastfeeding jaundice,
  • Breast milk jaundice
What is Physiologic jaundice and what causes it?

• Jaundice in healthy, full-term Neonates has been termed Physiologic because Hyperbilirubinemia occurs almost universally in neonates:

• Total serum bilirubin concentration usually peaks at 5 to 12 mg/dL on the Second or Third day after birth;

• Neonatal Physiologic jaundice may results from simultaneous occurrence of the following:
  • Bilirubin production is elevated because of increased RBC breakdown due to Shortened lifespan of Foetal RBC,
Hepatic excretory capacity is low because of:

- **Low** levels of binding protein **Ligandin** in Hepatocytes,
- **Low** activity of Conjugating enzyme (**UDP-GT)**,
- **Immaturity** of hepatic uptake and **Conjugating system**

In addition to these Physiologic considerations, Enterohepatic recirculation of bilirubin is relatively enhanced in Neonates;

- These factors can cause **Unconjugated Hyperbilirubinemia** in neonates;
- Bilirubin level usually becomes normal adult values at aged 2 - 3 weeks,
What is Non-physiologic or Pathologic jaundice?

Jaundice is Non-physiologic or Pathologic:

• If it occurs less than 24 hours after birth,
  • Jaundice in the first 24 hours after birth is not normal, and the causes of jaundice must be investigated,
• If increase in bilirubin is greater than 5.0mg/dL/ day;
• If total bilirubin exceed 15 mg/dL in a full-term infant or 10 mg/dL in a preterm infant;
• If Conjugated bilirubin concentration is very high;
• If evidence of acute hemolysis exists, or
• If hyperbilirubinemia persists beyond 10 days in a full-term or 21 days in a preterm infant;
What are some of the causes of Pathologic Unconjugated Hyperbilirubinemia?

- Increase bilirubin production via increased Hemolysis caused by either ABO or Rhesus Incompatibility;
- Rh Isoimmunization (Haemolytic disease of the Newborn, Erythroblastosis Fetalis) used to be a major cause of severe jaundice, often resulting in Kernicterus;
- Abnormalities of enzymes in RBC (e.g., G-6-PD deficiency) may cause increased hemolysis
• Defective hepatic uptake or conjugation due to:
  • Prematurity,
  • Hypoglycaemia,
  • Hypothyroidism,
  • Dehydration,
  • Bruising,
  • Polycythemia,
  • Inter-current Infection,
What is Breastfeeding Jaundice (Dehydration jaundice) and what causes it?

- **Breastfeeding jaundice** or **Dehydration jaundice** may develop in infants who breastfeed;
- It is due to Inadequate milk intake,
- It may occur on the 2\(^{nd}\) or 3\(^{rd}\) day of life, usually before commencement of milk production by the mother;
- Putting the infant to the breast more frequently and ensuring that the neonate latch-on properly to the breast may speed up milk production;
- Evaluation of the nutritional status and breastfeeding technique of the mother is essential for successful lactation and resolution of breastfeeding jaundice;
What is Breast Milk Jaundice?

- **Breast milk jaundice** manifests within the first 4 - 7 days of life and can persist for 3 - 12 weeks;
- It should be differentiated from Breastfeeding Jaundice, which occurs before the first 4 - 7 days of life and is due to Insufficient Production or Intake of Breast Milk;

**Breast milk jaundice:**
- Is elevation of Unconjugated bilirubin in Breastfed Newborn that develops in the first 4 - 7 days of life, 
- Persists beyond Physiologic jaundice, and
- Has no other identifiable cause;
What are some of the causes of Breast milk jaundice?

• Some possible causes of Breast milk jaundice:
  • Substance in breast milk that inhibits the Conjugating enzyme (UDP-GT) in liver of Neonates;
  • Lipoprotein Lipase in breast milk may produce non-esterified long-chain fatty acids that competitively inhibit UDP-GT;
    • Inhibition of UDP-GT causes unconjugated hyperbilirubinemia, leading to jaundice,
  • High level of Beta-Glucuronidase in breast milk may enhance de-conjugation of conjugated bilirubin in GIT of neonate, leading to increase Enterohepatic circulation of bilirubin, causing jaundice;
Other causes of Neonatal 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 the conjugating enzyme system (UDP-GT)
• **Conjugated Hyperbilirubinemia**: in Neonates it is usually Pathological; may be due to:
  
  • Developmental abnormalities of Biliary tree,
  
  • Obstructed bile flow with or without hepatocellular injury,
    
    • Extra-hepatic biliary Atresia, or
    
    • Intra-hepatic biliary Atresia
  
  • Neonatal Hepatitis:
    
    • May be due to: Infection, Metabolic (e.g., $\alpha_1$-Protease Inhibitor deficiency), Endocrine (e.g., Congenital Hypopituitarism);
PHOTOTHERAPY:

• Phototherapy is the primary treatment in neonates with unconjugated hyperbilirubinemia;
• It is effective because of changes in structure of bilirubin exposed to light;
• Unconjugated bilirubin is converted to water-soluble Photo-isomer Lumirubin,
• Lumirubin is excreted in bile and in urine;
• It is mostly responsible for the therapeutic effect of phototherapy of lowering the serum bilirubin level;
REFERENCES

• http://www.emedicine.com/PED/topic2774.htm
• http://www.emedicine.com/med/topic1066.htm
• http://www.emedicine.com/PED/topic282.htm