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MBBS YEAR 4

OVERVIEW OF: NEONATAL Hb, O<sub>2</sub>-TRANSPORT; JAUNDICE

**Give a brief description of Haemoglobin (Hb):**

- Hb is made up of Four Subunits (Tetramer) held together by multiple non-covalent interactions
- Each subunit (Monomer) consist of: Haem (Ferro-Protoporphyrin) and Globin protein
- Haem consist of a Protoporphyrin IX and Ferrous ion (Fe<sup>2+</sup>)
- Globin protein folds around the Haem group forming a hydrophobic pocket that protects the Haem, which serves as the site of Oxygen binding
- Different types of Hb (Hb F, Hb A) are present in humans
- Primary structure of Globin protein is the major difference in the types of Hb;
- Different subunits of various types of 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
- **Figure 1** is Schematic diagram of time of expression of Genes for Hb subunits during development
  - **Embryogenesis:**
    - Initial Hb type formed is a Tetramer of **Two Zeta (ζ) subunits**, (which are evolutionally similar to **α subunits**), and **Two Epsilon (ε) subunits** (ζ<sub>2</sub> ε<sub>2</sub>)
  - Through **First Six months of development:**
    - Zeta (ζ) subunits are replaced by **Alpha (α) subunits**, and
    - Epsilon (ε) subunits are replaced by **Gamma (γ) subunits** forming **Haemoglobin F (α<sub>2</sub> γ<sub>2</sub>)**
  - Through later embryonic development and just after birth:
    - **Beta (β) subunits** replaced **Gamma (γ) subunits**
    - Forming **α<sub>2</sub> β<sub>2</sub> (Hb A) or Adult Hb**
  - Compared to Adult Hb (α<sub>2</sub> β<sub>2</sub>) Foetal Hb (Hb F) is the major Haemoglobin in Neonates
  - Hb F is made up of **Two γ-chains** in place of **Two β-chains** in adult Hb and is represented as α<sub>2</sub> γ<sub>2</sub>
  - Shortly before birth **γ-chain** synthesis diminishes and **β-chain** synthesis is initiated
  - By age of between Six and Seven months over 90% of Infant's haemoglobin is Hb A

Two types of Hb A:

- **Hb A<sub>1</sub>** (α<sub>2</sub> β<sub>2</sub>) is the major (98%) form of Hb A in human adults;
- **Hb A<sub>2</sub>** (α<sub>2</sub> δ<sub>2</sub>) is a minor (2%) form of Hb A in human adults;

### How is the structure of Hb related to functions (Structure – Function relationships)?

- ❑ Mechanism of Cooperative binding of Oxygen to Hb and the fact that Protons ( $H^+$ ),  $CO_2$ , and 2,3-BPG are allosteric effectors of Hb emphasizes the role structure plays in the function of Hb (**Figs. 2a, 2b, and 2c**)
- ❑ Hb F is adapted to the environment of the Foetus that gets  $O_2$  from Maternal blood
- ❑ Foetus must pick up  $O_2$  at the Low partial pressure of  $O_2$  of the Placenta,
  - Structure of Hb F must be different from that of Maternal Hb that releases the  $O_2$  in the placenta
  - Affinity of Hb F for  $O_2$  must be Higher than that of Hb A for  $O_2$
- ❑ Higher affinity of Hb F for  $O_2$  is because  $\gamma$ -subunits in Hb F does not bind 2,3-BPG well
- ❑ Implies that  $O_2$ -binding curve of Hb F is shifted to the Left of Hb A<sub>1</sub> (Fig. 3)
- ❑ About 15 to 20% of Hb F is acetylated at the N-terminals (Hb F<sub>1</sub>)
- ❑ Hb F<sub>1</sub> 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, combined with increase RBC concentration of 2,3-BPG that peaks three months after birth, results in a gradual shift to the Right of the Infant's Oxygen-binding curve
- ❑ Overall result is greater delivery of Oxygen to Tissues at this stage than at birth, in spite of a 30% decrease in the infant's total Hb level

### BILIRUBIN METABOLISM:

#### Briefly outline metabolism of Bilirubin from Hb?

- ❑ Bilirubin produced in Reticuloendothelial system during Haem catabolism
- ❑ Heme Oxygenase catalyzes Heme to Biliverdin, Iron and Carbon Monoxide
- ❑ Carbon Monoxide is excreted via Lungs and can be measured in patient's breath to quantify bilirubin production
- ❑ Biliverdin is reduced to Unconjugated Bilirubin, which is hydrophobic
- ❑ Unconjugated bilirubin is transported in plasma bound mainly to Albumin,
- ❑ Binding of bilirubin to albumin increases Postnatal with age and is reduced in sick infants
- ❑ Presence of endogenous and exogenous binding competitors (certain drugs) decreases binding affinity of Albumin for bilirubin
- ❑ Small fraction of unconjugated bilirubin in plasma not bound to albumin can cross cell membrane, including blood-brain barrier, leading to Neurotoxicity
- ❑ Bilirubin-Albumin complex transported into Hepatocytes and binds to **Ligandin**
- ❑ Ligandin concentrations are low at birth, but increase rapidly over the first few weeks of life
- ❑ Pharmacologic agents (e.g., Phenobarbital) increase concentration Ligandin
- ❑ UDP-Glucuronyl-Transferase (UDP-GT) catalyzes Conjugation of Bilirubin in Hepatocytes
- ❑ Conjugated Bilirubin is soluble in aqueous medium, thus it is 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 be administered to increase UDP-GT activity
- ❑ Conjugated Bilirubin in bile is transferred to Intestines,
- ❑ Some Conjugated Bilirubin are metabolised by Microbes in Colon to form colourless compounds (Mesobilinogens or Urobilinogens)
- ❑ Some De-conjugation occurs in Proximal Small Intestine through the action of  **$\beta$ -glucuronidases** located in Brush Border
- ❑ Unconjugated bilirubin formed is reabsorbed into Circulation, increasing the total plasma Bilirubin pool
- ❑ Cycle of Uptake, Conjugation, Excretion, De-conjugation, and Reabsorption is termed **Enterohepatic Circulation of Bilirubin, which occurs mainly in Neonates**

### How is bilirubin cleared from Foetal blood? (Prenatal Clearing of bilirubin):

- ❑ Unbound Foetal bilirubin continuously crosses Placenta into the Mother because of greater albumin-binding capacity of Maternal plasma
- ❑ Bilirubin from Foetus is Conjugated in Maternal Liver by UDP-Glucuronyl-transferase (UDPGT)
- ❑ UDP-GT in healthy mothers is in great excess so that even by-products of major haemolytic events do not exceed the capacity of maternal UDPGT to conjugate bilirubin
- ❑ Conjugated bilirubin now formed in maternal blood is secreted by active transport into the bile canaliculi and becomes concentrated in Gallbladder before excretion into intestinal tract
- ❑ Enterohepatic circulation of bilirubin can occur in some cases
  - De-conjugation and re-sorption of bilirubin is minimal in healthy adults because of the action of Intestinal bacteria, which metabolises the conjugated bilirubin to Stercobilinogen and Urobilinogen that are excreted in the maternal stool

### How is bilirubin metabolised in Neonate? (Neonatal Metabolism of Bilirubin):

- ❑ Conjugation of Bilirubin is limited in the Liver of Foetus and Neonate, because of Immaturity of Conjugating enzyme system (UDPGT)
- ❑ At birth: UDPGT in liver of Neonate is about 0.1% to 1.0% that of adult
- ❑ UDPGT increases over time but does not reach adult levels until 6 to 14 weeks after birth
- ❑ Daily load of bilirubin excretion in Neonate is disproportionately large
  - Thus, bilirubin accumulates in bloodstream of all Neonates
- ❑ A twofold increase in neonatal bilirubin production occurs because of:
  - Higher circulating erythrocyte volume and,
  - Change in the life span of the erythrocyte
- ❑ Intestinal Beta-Glucuronidase is high in Neonates, resulting in increased de-conjugation of conjugated bilirubin, and consequently in much greater re-sorption of unconjugated bilirubin through the **Enterohepatic circulation** of bilirubin
- ❑ Certain factors in Breast-milk of some mothers contribute to increased Enterohepatic circulation of bilirubin (breast-milk jaundice)
  - This is especially true of 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, with the result that both conjugated bilirubin 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, such as:
  - 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: Such factors include:
    - Acid – Base disturbances
    - Presence of drugs (e.g., Sulphonamides)

- ❑ Hyperbilirubinemia in Neonates is Primarily due to Immaturity of the Conjugating enzyme system (UDPGT) in the Liver of Neonates
- ❑ Severe unconjugated hyperbilirubinemia in neonates could result in **Kernicterus** (bilirubin encephalopathy):
  - Staining of Basal Ganglia by unconjugated bilirubin and involves diffuse neuronal damage causing severe neurologic consequences

### Neonatal Jaundice:

- ❑ Jaundice in Neonates can be classified as 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, including:
  - 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 newborns 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 result from simultaneous occurrence of the following:
  - Bilirubin production is elevated because of increased red cell breakdown due to Shortened lifespan of Foetal red cells and higher
  - Hepatic excretory capacity is low because of :
    - **Low** concentrations of binding protein **Ligandin** in Hepatocytes,
    - **Low** activity of Conjugating system (**UDP-GT**)
    - **Immaturity** of hepatic update and **Conjugating system**
  - In addition to these Physiologic considerations, Enterohepatic recirculation of bilirubin is relatively enhanced in Neonates
  - These factors can lead to Unconjugated Hyperbilirubinemia, which typically become normal adult values when the neonate is aged 2 - 3 weeks

### What is Non-physiologic or Pathologic jaundice?

Jaundice should be considered 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 the jaundice must be investigated
- ❑ If bilirubin levels rise at a rate of greater than 5.0 mg/dL per day,
- ❑ If total bilirubin levels exceed 15 mg/dL in a full-term or 10 mg/dL in 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

**TAKE NOTE:**

- ❑ Breast-feeding is an important risk factor for hyperbilirubinemia, probably due to high levels of Beta-Glucuronidase in breast milk.

**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) use to be a major cause of severe jaundice, often resulting in Kernicterus
    - ❑ Unlike most developing countries, Rh Isoimmunization is now relatively rare in developed countries that uses Rh prophylaxis in Rh-negative women
- ❑ Abnormalities of enzymes in RBC (e.g., G-6-PD deficiency) may cause increased hemolysis
- ❑ Defective hepatic uptake or conjugation may be due to either:
  - ❑ Prematurity, Hypoglycaemia or Hypothyroidism
- ❑ Dehydration, Bruising, Polycythemia, Intercurrent Infection

**What is Breastfeeding Jaundice (Dehydration jaundice) and what causes it?**

- ❑ Physiologic jaundice can be accentuated by breastfeeding, which in the first few days of life results in lower calorie intake, especially if milk production starts late
- ❑ **Breastfeeding jaundice** or **Dehydration jaundice** may develop in infants who breastfeed
- ❑ Breastfeeding jaundice is usually due to Inadequate milk intake,
  - Breastfeeding jaundice may occur on the 2<sup>nd</sup> or 3<sup>rd</sup> 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 overall 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
- ❑ Breast milk jaundice should be differentiated from **Breastfeeding Jaundice**, which occurs before the first 4 - 7 days of life and is caused by Insufficient Production or Intake of Breast Milk
- ❑ Specifically Breast milk jaundice:
  - Is an elevation of Unconjugated bilirubin in a Breastfed Newborn that develops following the first 4 - 7 days of life,
  - Persists beyond Physiologic jaundice, and
  - Has no other identifiable cause

**What are some of the possible causes of Breast milk jaundice?**

- ❑ Some possible causes of Breast milk jaundice include:
  - Substance in breast milk that inhibits the Conjugating enzyme system, (UDP-GT) in liver of Neonate
  - Lipoprotein Lipase in breast milk may produce non-esterified long-chain fatty acids that competitively inhibit UDP-GT
    - ❑ Inhibition of UDP-GT results in unconjugated hyperbilirubinemia, leading to jaundice

- High levels of Beta-glucuronidase in breast milk may enhance de-conjugation of conjugated bilirubin in GIT of neonate, leading to increase enterohepatic circulation of bilirubin, results in jaundice

### Other causes of Neonatal Jaundice:

#### Unconjugated Hyperbilirubinemia:

- Inherited disorders of Bilirubin metabolism leading to decreased clearance of bilirubin (Examples include):
  - **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, Conjugated Hyperbilirubinemia is always pathological
- Conjugated Hyperbilirubinemia can 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) etc.

#### Phototherapy:

- Phototherapy is the primary treatment in neonates with unconjugated hyperbilirubinemia
- Phototherapy is effective because of changes that occur when bilirubin is exposed to light
  - Although bilirubin is bleached through the action of light, the process is slow and is now believed to contribute only minimally to the therapeutic effect of phototherapy
  - Configurational Isomerization is a very rapid process that changes some of the predominant bilirubin isomers to water-soluble Photo-isomers such as **Lumirubin**, which forms between 2 – 6% of the total serum bilirubin concentration
  - Lumirubin is the primary pigment found in bile during phototherapy
  - Lumirubin is excreted in bile and, to some extent, in urine; It is mostly responsible for the therapeutic effect of phototherapy of lowering the serum bilirubin level

#### Reference:

1. <http://www.emedicine.com/PED/topic2774.htm>
2. <http://www.emedicine.com/med/topic1066.htm>
3. <http://www.emedicine.com/PED/topic282.htm>