NEONATAL Hb, O_2-TRANSPORT & JAUNDICE: OVERVIEW

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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 (ζ_2 ε_2):
- **Two Zeta** (ζ) subunits, (which are evolutionally similar to α subunits,) and
- **Two Epsilon (ε)** 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);**

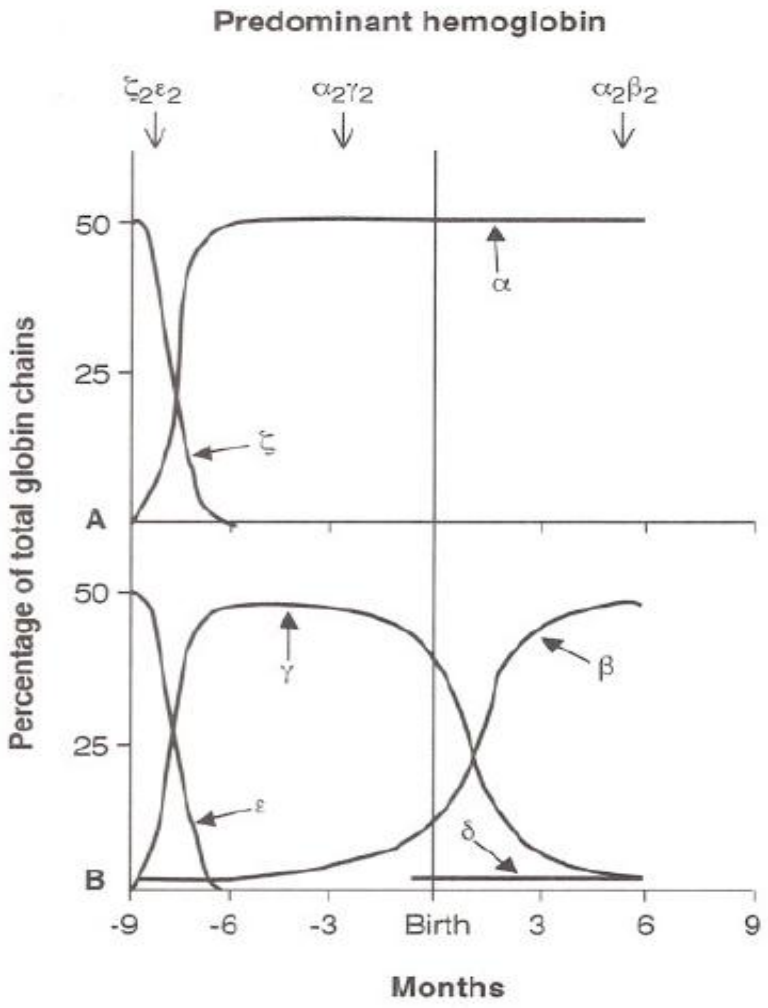
- Through later embryonic development and shortly before birth **γ-chain** synthesis diminishes and **β-chain** synthesis is initiated;
- Thus, **Beta (β) subunits** replaced **Gamma (γ) subunits;**
- Forming $\alpha_2\ \beta_2$ **Adult Hb (Hb A);**
- Compared to Adult Hb ($\alpha_2\ \beta_2$) Foetal 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 A_1** (α_2 β_2) is the major (98%) form of Hb A in adults;
- **Hb A_2** (α_2 δ_2) is a minor (2%) form of Hb A in adults;

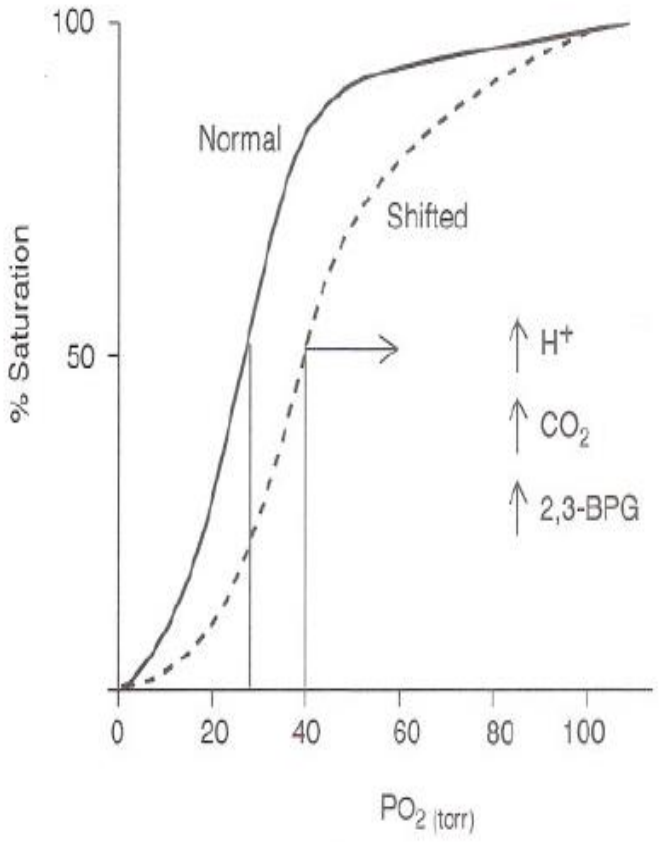
Fig. 1: Diagram of expression of genes for different Globin chains in Hb during early development (Davidson & Sittman: Biochemistry 3rd Ed)



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

- Mechanism of Cooperative binding of O_2 to Hb, allosteric effects of H^+, CO_2 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 O_2 from Maternal blood;
- Foetus must pick up O_2 at the Low pO_2 of the Placenta,
- Structure of Hb F is different from Maternal Hb that releases the O_2 in the placenta;
- Affinity of Hb F for O_2 is Higher than that of Hb A for O_2
- Higher affinity of Hb F for O_2 is because **γ-subunits** does not bind 2,3-BPG well;

Figs 2a, 2b: Action of allosteric effectors on Oxygen binding curve of HbA,



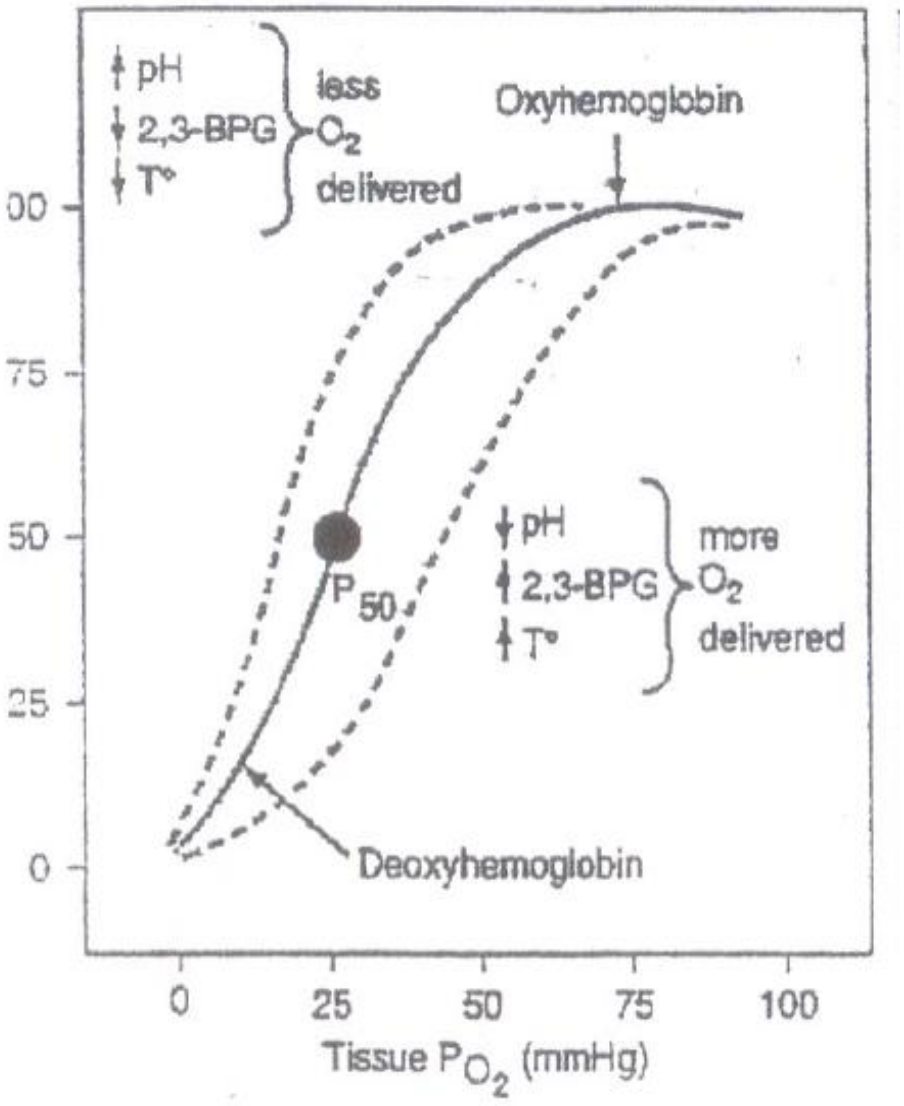
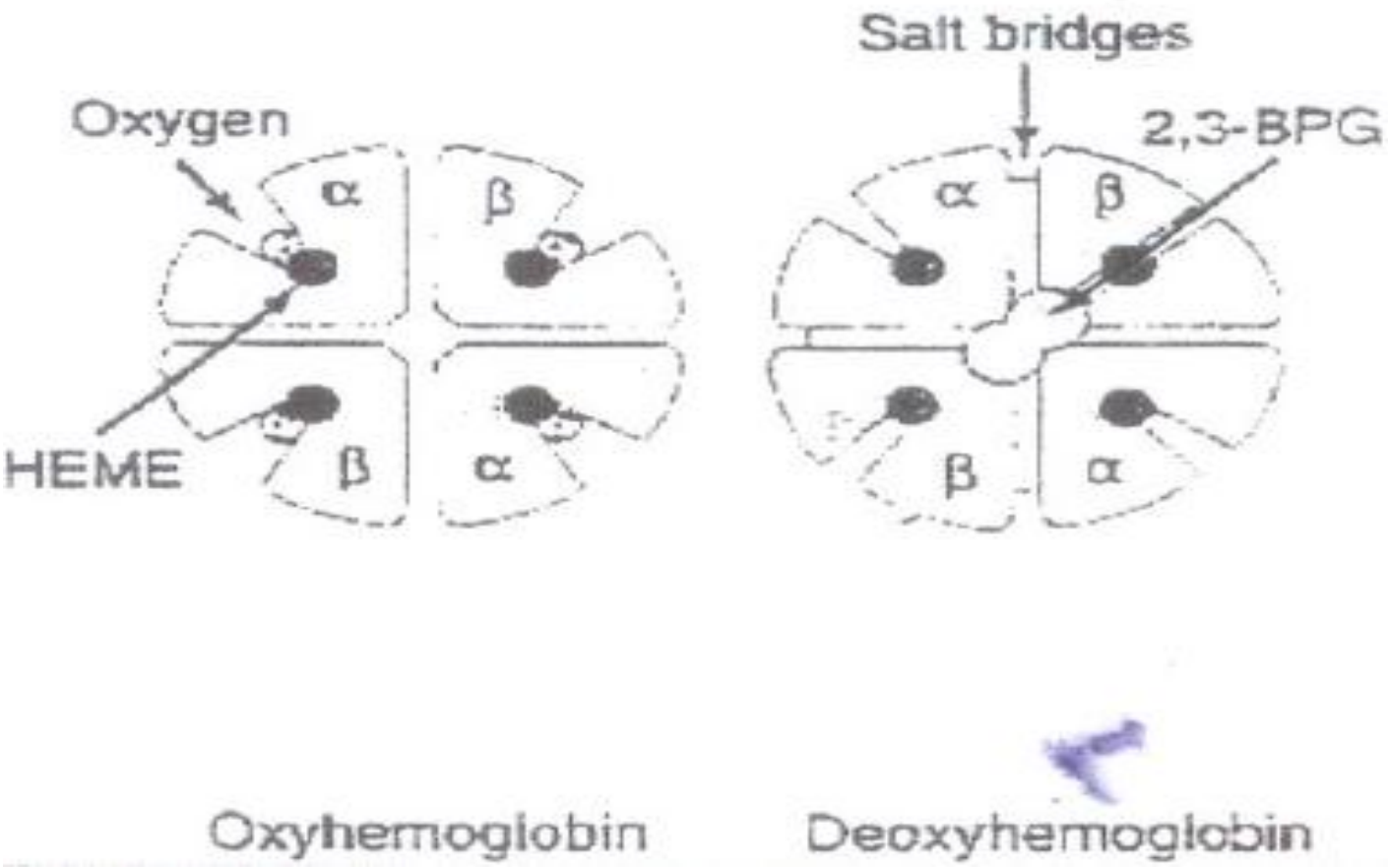
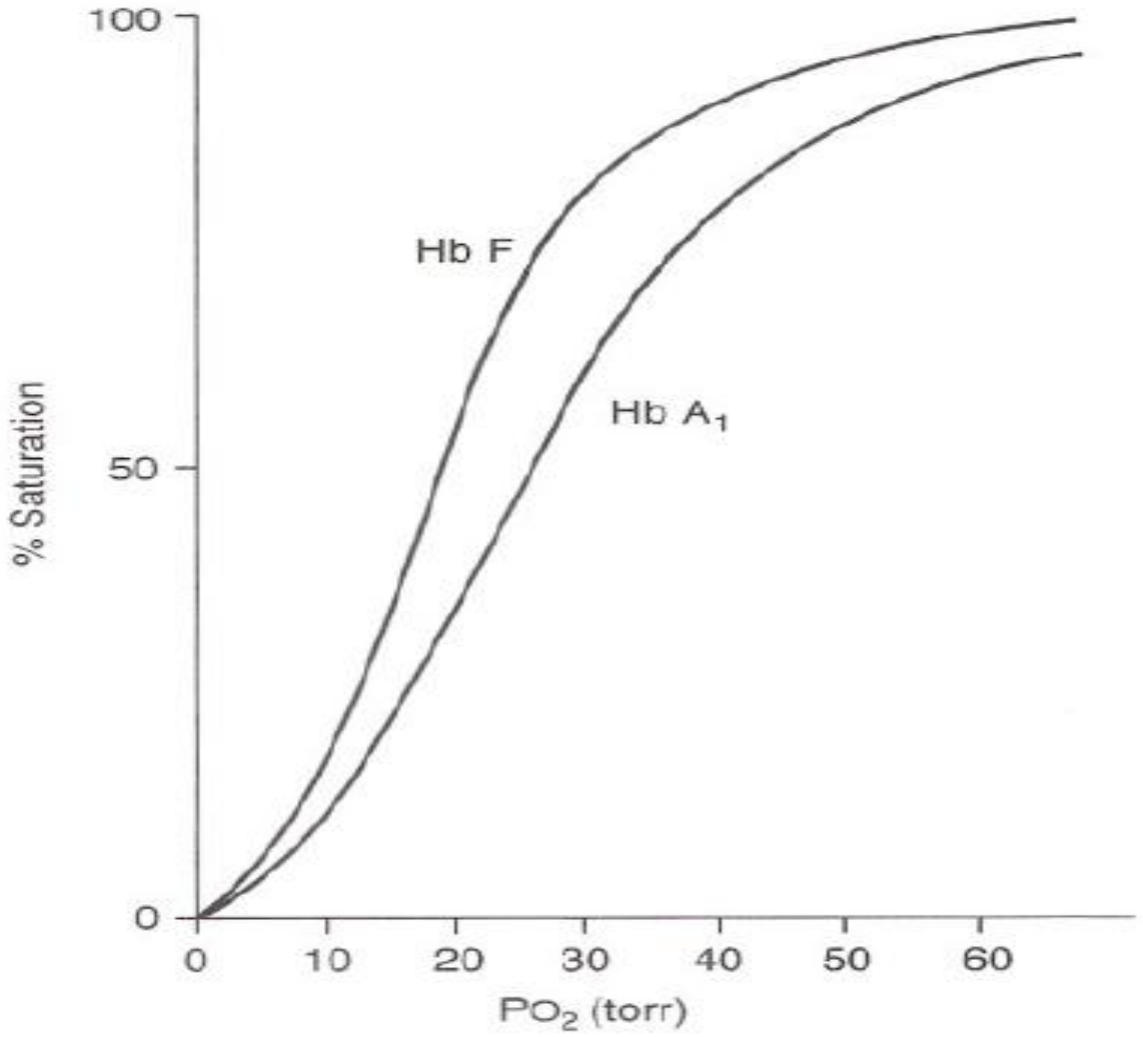


Fig. 3: Diagram of binding of 2,3-BisPhosphoglycerate (2,3-BPG)



- 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,

NEONATAL JAUNDICE

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) use 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 **2nd or 3rd** 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., α_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;

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