INTRODUCTION

Hyperbilirubinemia is the most common condition that requires medical attention in newborns. The yellow coloration of the skin and sclera in newborns with jaundice is the result of accumulation of unconjugated bilirubin. In most infants, unconjugated hyperbilirubinemia reflects a normal transitional phenomenon. However, in some infants, serum bilirubin levels may excessively raise, which can be cause for concern **(Hansen et al., 2015).**

Indirect bilirubin, the main cause of neonatal jaundice, is strongly neurotoxic for the undeveloped neural system, especially when the indirect bilirubin concentration exceeds the albumin binding capacity. Unconjugated bilirubin binds the phospholipids of neuronal plasma membranes as well as the phospholipids of subcellular organelles membrane, which leads to cell oxygen deprivation, energy metabolism impairment, and cell death. The phenomenon of deposited indirect bilirubin in basal ganglia as well as in the vestibule-cochlear nucleus causes a neurological syndrome called kernicterus as well as sensorineural hearing loss **(Thilo and Rosenberg, 2011).**

Alteration in the equilibrium between bilirubin production, conjugation, and excretion cause this transitional elevation during the neonatal period. The pathophysiology of this condition may vary according to the etiology **(Tioseco et al., 2005).**

Bilirubin toxicity remains a significant problem despite recent advances in the care of jaundiced (hyperbilirubinemic) neonates. There is a recent increase in reported cases of classical kernicterus, partly due to earlier hospital discharge. New reports of hyperbilirubinemia-induced auditory dysfunction using hearing screening and evoked potential testing, underscore the need for better understanding of how hyperbilirubinemia causes brain damage in some infants, especially because the damage is preventable **(Steven and Shapiro, 2003).**

The most common causes of pathologic indirect hyperbilirubinemia are: increased bilirubin production due to hemolytic diseases which includes immune-mediated hemolysis (ABO or Rh incompatibility), inherited red cell membrane defects (spherocytosis), erythrocyte enzymatic defects (glucose-6-phosphate dehydrogenase (G6PD) deficiency), and sepsis, decreased clearance (Crigler-Najjar and Gilbert's syndromes) and increased enterohepatic circulation such as breast feeding jaundice **(Najib et al,. 2013).** Hyperbilirubinemia at neonatal period is one of the major deteriorating factors of the auditory system; if left untreated, it leads to cerebral damage **(Baradaranfar et al., 2011).**

Currently, the most sensitive means of assessing neurotoxicity may be auditory brain stem evoked response (ABR), which shows the predictable early effects of bilirubin toxicity **(Thilo and Rosenberg, 2011).**

Auditory Brainstem Response (ABR) offers a noninvasive modality to assess neural integrity of the auditory pathway. It is an effective and simple method that requires less cooperation of the patient and measures the specific part of the auditory pathway. It is not significantly altered by state of consciousness, drugs, or environmental factors **(American Academy of Pediatrics, 2004).**

ABR used to detect auditory neuropathy or neural conduction disorders in newborns. Because ABR are reflective of auditory nerve and brainstem function, these infants can have an abnormal ABR screening result even when peripheral hearing is normal **(Kehrle et al., 2008)**.

Bilirubin can produce behavioral changes and alterations in ABR at total serum bilirubin (TSB) below 20 mg/dl. As the TSB increases, changes in ABR progress from small increases in interwave intervals to decreased amplitude and eventual loss of waves III and V, this represents altered brainstem conduction. Wave I abnormalities, which reflects auditory nerve function, may be seen at higher bilirubin levels and kernicteric infants with hearing loss **(Wennberg et al., 2006).**

Discriminating between benign and serious causes of jaundice is a common task faced by most pediatricians and neonatologists in their daily practice **(Karpen, 2007).**

Even a mild hearing loss can cause significant speech disorder and hold up development of communication skills **(Okhravi et al., 2015)**.

Regardless of the cause of indirect hyperbilirubinemia, the goal of the therapy is to prevent bilirubin related neurotoxicity. Phototherapy and, if unsuccessful, exchange transfusion remain the primary treatment modalities used to keep the maximal total serum bilirubin below the pathologic levels **(Piazza and Stoll, 2007).**

Early diagnosis and treatment of hyperbilirubinemia is highly important for preventing hearing loss and all newborns with pathologic hyperbilirubinemia must be screened. ABR abnormalities may be transient in majority of patients **(Okhravi et al., 2015).**

AIM OF THE WORK

This study aims to determine the effect of neonatal hyperbilirubinemia on auditory brainstem response (ABR) and to evaluate the effect of treatment of hyperbilirubinemia on ABR findings.

NEONATAL JAUNDICE

Neonatal jaundice or **Neonatal hyperbilirubinemia**, is a yellowing of the skin and other tissues of a newborn infant. A bilirubin level of more than 5 mg/dl manifests clinical jaundice in neonates whereas in adults a level of 2mg/dl would look icteric **(Hansen et al., 2015).** As the Total serum bilirubin (TSB) level of healthy term infants peaks at approximately 5 mg/dl, a more useful definition of hyperbilirubinemia might be a TSB level that exceeds the 95th percentile for the infants' age in hours **(Maisels, 2006)**.

Neonatal jaundice is one of the most common neonatal problems. Although up to 60% of term newborns have clinical jaundice in the first week of life, few have significant underlying disease **(Balistreri, 2004).**

However, hyperbilirubinemia in neonatal period can be associated with severe illness such as hemolytic disease, metabolic and endocrine disorders, anatomic abnormalities in the liver and infections **(Balistreri and Bezerra, 2006).**

Frequency

Neonatal hyperbilirubinemia is extremely common because almost every newborn develops an unconjugated serum bilirubin level of more than (1.8 mg/dl) during the first week of life. About 65% to 75% of neonates have clinical jaundice **(Hansen et al., 2015).**

Incidence figures are difficult to compare because authors of different studies do not use the same definitions for significant neonatal hyperbilirubinemia or jaundice. In addition, identification of infants to be tested depends on visual recognition of jaundice by health care providers, which varies widely and depends both on observer attention and on infant characteristics such as race and gestational age **(Hansen et al., 2015).**

Over the years, it has been documented that there is increase in reported cases of hyperbilirubinemia. This increase could be due to more awareness, better laboratory methods and increase in incidence of breast feeding. Incidence is higher in infants who are breastfed or who receive inadequate nutrition. The mechanism for this phenomenon may not be fully understood. However, when inadequate feeding volume is involved, increased enterohepatic circulation of bilirubin probably contributes to prolonged jaundice.The incidence is much higher in preterm neonates **(Kumral et al., 2009).**

- **Neonatal Bilirubin Metabolism**

Bilirubin is one of the biologically active end products of heme catabolism fig (1) **(Kaplan et al., 2011).**

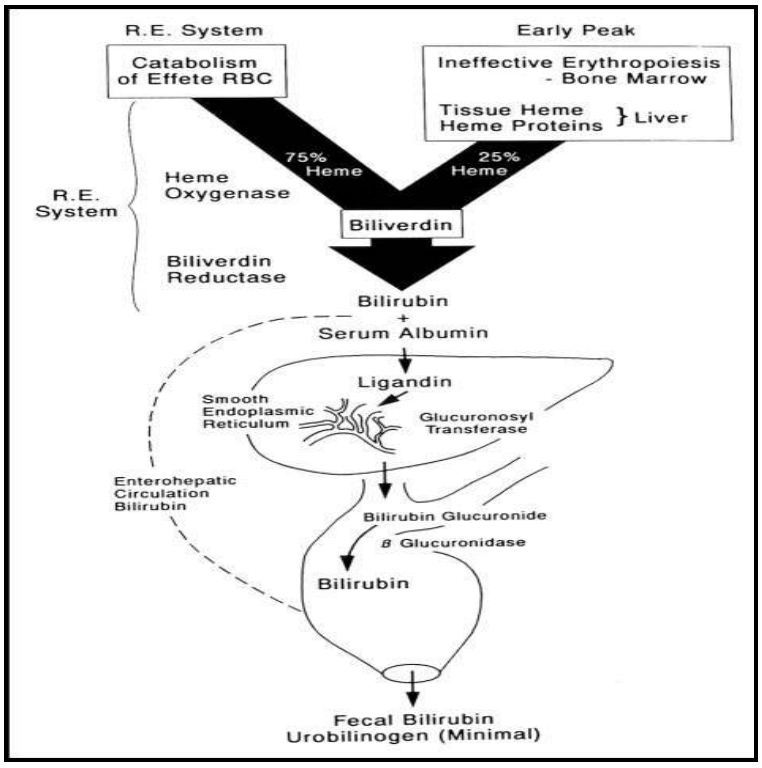

Figure (1): Neonatal bilirubin metabolism "Maisels MJ (2005). In: MacDonald MG, Mullett MD, Seshia MMK, eds. Avery's neonatology: pathophysiology and management of the newborn. Philadelphia: Lippincott Williams and Wilkins; 768-846."

- **Bilirubin Formation:**

Bilirubin is produced in the reticuloendothelial system as the end product of heme catabolism and is formed through oxidation-reduction reactions. Approximately 75% of bilirubin is derived from hemoglobin, but degradation of myoglobin, cytochromes, and catalase also contributes**.** In the first oxidation step, biliverdin is formed from heme in adenosine triphsphate-dependant reaction through the action of heme oxygenase releasing iron and carbon monoxide. Heme

oxygenase is the rate –limiting enzyme in bilirubin production. It allows for the degradation of heme from hemoglobin to form biliverdin **(Hansen et al., 2015)**. This process is energy requiring because NADPH donates electrons through the cytochrome system and molecular oxygen consumed for liberation of iron from the porphyrin ring of heme, the release of carbon monoxide, as well as the formation of biliverdin **(Dennery et al., 2001).**

The reaction produces a molecule of carbon monoxide for every molecule of biliverdin **(Shapiro, 2003).** The iron conserved for reuse, whereas carbon monoxide is excreted through the lungs and can be measured in the patient's breath to quantify bilirubin production. Next, water-soluble biliverdin is reduced by biliverdin reductase to unconjugated bilirubin, which, because of the intermolecular hydrogen bonds, is almost insoluble in water in its most common isomeric form (fig.2) **(Hansen et al., 2015)**.

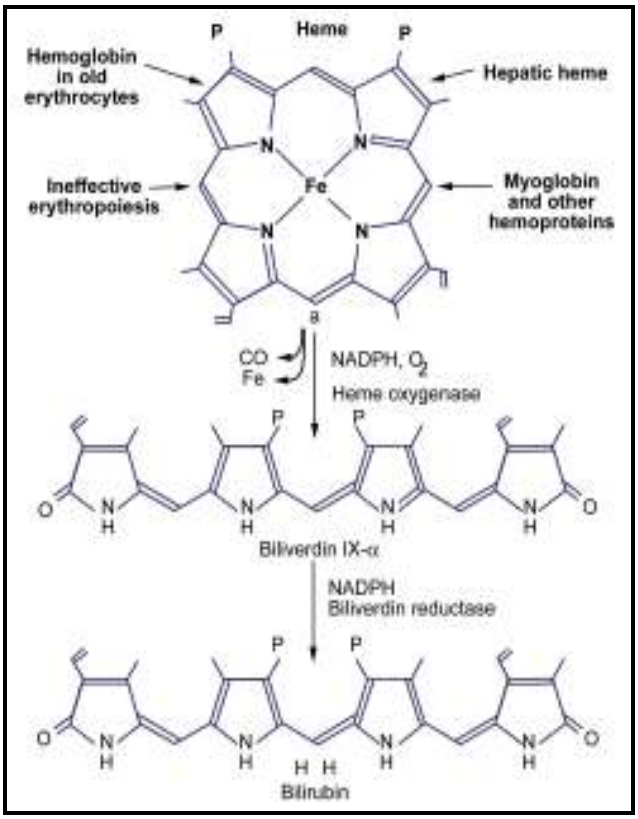

Figure (2): Bilirubin Formation "Maruhashi T, Soga J, Fujimura N, et al (2012): Hyperbilirubinemia, augmentation of endothelial function, and decrease in oxidative stress in Gilbert syndrome. Circulation; 126(5):598-603"

The unconjugated bilirubin is a potent natural antioxidant at low levels, but neurotoxic at high levels **(Shapiro, 2003).**

The process occurs in all nucleated cells except mature a nucleated red blood cell (RBCs), which lack this enzymatic system for degrading heme. The reticuloendothelial system, in particular the spleen, is important in degrading heme from hemoglobin-containing RBCs **(Wong et al., 2007).** Catabolism of 1 gram of hemoglobin; result in the production of 35 mg of

bilirubin **(Gregory et al., 2012)**. Combined with the shortened lifespan of the neonatal RBCs, the potential bilirubin production load is significant. Also, in fetal life, bilirubin production begins as early as 12 weeks of gestation, with the major route of elimination through the placenta. Glucuronidase in the fetal bowel enables bilirubin to remain unconjugated and reabsorbed for maternal excretion. The presence of some of these fetal mechanisms in the neonatal period can contribute to neonatal hyperbilirubinemia after birth **(Reiser, 2004)**.

- **Bilirubin Transport:**

After formation, bilirubin diffuses into the circulation. In the absence of conjugation, the total bilirubin concentration in plasma is the sum of bilirubin bound to albumin plus a minimal amount of free bilirubin **(Wang et al., 2007).** At this stage, bilirubin is lipid soluble and unconjugated. Unconjugated bilirubin binds to albumin **(Moerschel et al., 2008).**

Because of its hydrophobic nature, unconjugated bilirubin transported in the plasma tightly bound to albumin. Binding to other proteins and erythrocytes also occurs, but the physiologic role is probably limited. Binding of bilirubin to albumin increases postnatally with age and is reduced in infants who are ill **(Hansen et al., 2015).**

The presence of endogenous and exogenous binding competitors, such as certain drugs, also decreases the binding

affinity of albumin for bilirubin. A minute fraction of unconjugated bilirubin in serum is not bound to albumin. This free bilirubin is able to cross lipid-containing membranes, including the blood-brain barrier, leading to neurotoxicity **(Moerschel et al., 2008).**

When it reaches the liver, bilirubin transported into liver cells, where it binds to ligandin. Uptake of bilirubin into hepatocytes increases with increasing ligandin concentrations. Ligandin concentrations are low at birth but rapidly increase over the first few weeks of life. Ligandin concentrations may be increased by the administration of pharmacologic agents such as phenobarbital **(Gregory et al., 2012).**

- **Bilirubin Conjugation:**

Bilirubin is bound to glucuronic acid (conjugated) in the hepatocyte endoplasmic reticulum in a reaction catalyzed by uridin diphosphoglucuronyl transferase (UDPGT) (fig.2). Monoconjugates are formed first and predominate in the newborn. Diconjugates appear to be formed at the cell membrane and may require the presence of the UDPGT tetramer (fig 3) **(Hansen et al., 2015).**

Bilirubin conjugation is biologically critical because it transforms a water-insoluble bilirubin molecule into a water-soluble molecule. Water-solubility allows conjugated bilirubin to be excreted into bile. UDPGT activity is low at birth but increases to adult values by age 4-8 weeks. In addition, certain

drugs (phenobarbital) can be administered to increase UDPGT activity **(Hansen et al., 2015).**

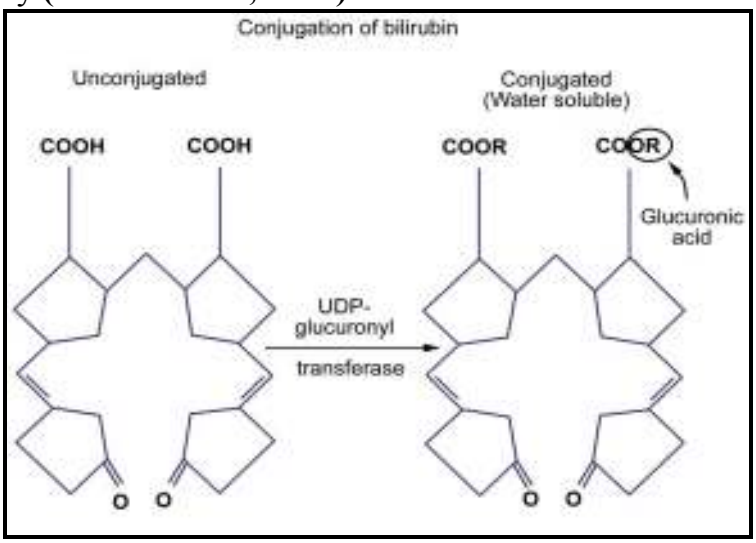

Figure (3): Bilirubin Conjugation "Maruhashi T, Soga J, Fujimura N, et al (2012): Hyperbilirubinemia, augmentation of endothelial function, and decrease in oxidative stress in Gilbert syndrome. Circulation; 126(5):598-603"

Bilirubin Excretion:

Bilirubin excreted more slowly in the newborn compared with the adult because of a transiently slower rate of hepatic uptake free bilirubin from the blood and decreased concentrations of UDPGT. As free bilirubin uptake and conjugation improve over the first 2 weeks after birth, increasing amounts of conjugated bilirubin enter the bile ducts and are excreted with the bile into the intestinal tract **(Wang et al., 2007).**

Once excreted into bile and transferred to the intestines, bilirubin is eventually reduced to colorless tetrapyrroles by

microbes in the colon. However, some deconjugation occurs in the proximal small intestine through the action of B-glucuronidases located in the brush border. This unconjugated bilirubin can be reabsorbed into the circulation, increasing the total plasma bilirubin pool. This cycle of uptake, conjugation, excretion, deconjugation, and reabsorption is termed 'enterohepatic circulation'. The process may be extensive in the neonate, partly because nutrient intake is limited in the first days of life, prolonging the intestinal transit time **(Hansen et al., 2015).**

The meconium accumulating in the gut in utero also contains about 100 to 200 mg of bilirubin per 100 gm of meconium at birth, of which 50% or more is unconjugated. Thus the miscible pool of bilirubin is affected not only by the relative rates of bilirubin production and removal from the circulation, but also the rates of enterohepatic circulation and meconium excretion **(Wang et al., 2007).**

- **Pathogenesis of hyperbilirubinemia**

Neonatal hyperbilirubinemia results from excessive production of bilirubin and the limited ability to excrete it **(Maisels and Kring, 2006).**

Newborn infants have several factors that increase their risk of developing physiologic jaundice or high serum bilirubin concentrations in the first days of life.

First, unconjugated bilirubin not readily excreted in newborn infants and the ability to conjugate bilirubin is limited**.** Infants have immature UDPGT, which causes an increase in unconjugated bilirubin **(Dennery et al., 2001)**.

Second, newborn infants have red blood cells with a decreased life span causing an increase in hemoglobin, which produces bilirubin at a higher rate than adults **(Shapiro, 2003).**

The immature UDPGT and increased hemoglobin are responsible for physiologic jaundice of the neonate **(Shapiro, 2003).** Jaundice usuallybecome apparent when total serum bilirubin levels exceed 5mg/dl. Jaundice can be classified as physiologic or pathologic according to post-delivery timing of onset, clinical course, resolution, rate of bilirubin increases, and total serum bilirubin levels **(Melton and Akinbi, 1999).**

Apparently healthy, term newborns, with TSB levels > 20mg/dl don't have physiologic jaundice. They have hyperbilirubinemia, in time, and with better techniques, we might identify the cause of jaundice in more of these infants **(Maisels, 2006).**

Physiologic jaundice is caused by a combination of increased bilirubin production secondary to accelerated destruction of erythrocytes, decreased excretory capacity secondary to low levels of ligandin in hepatocytes, and low activity of the bilirubin-conjugating enzyme uridine diphosphoglucuronyl transferase (UDPGT) **(Hansen et al., 2015).**