Audiological findings in infants and toddlers with hyperbilirubinemia and physiological jaundice: A longitudinal study

Reg. No. A0390009

A Master's Dissertation submitted in part fulfillment of Master of Science (Audiology) University of Mysore, Mysore

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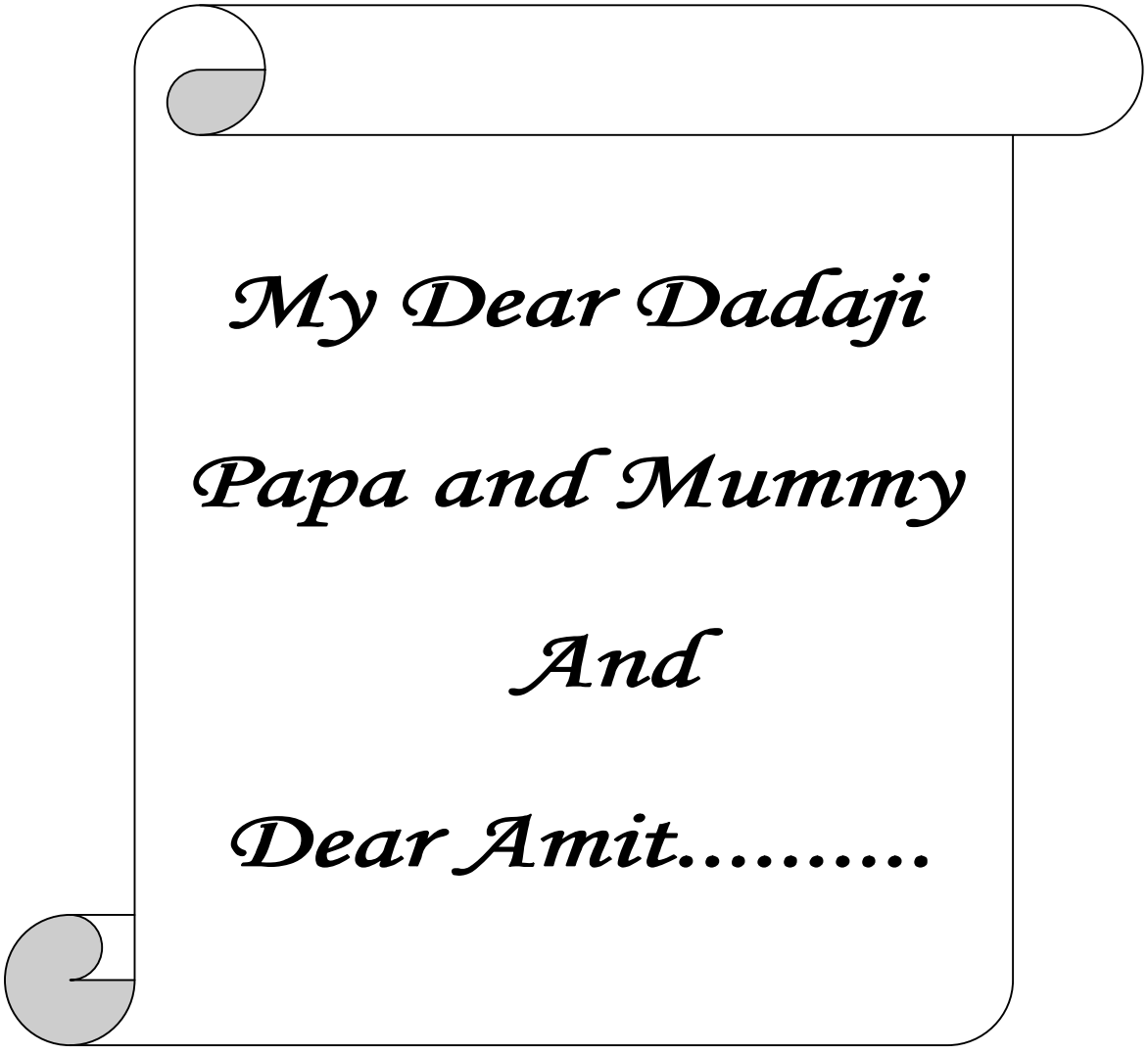
Dedicated to.....



CERTIFICATE

This is to certify that this dissertation entitled **"AUDIOLOGICAL FINDINGS IN INFANTS AND TODDLERS WITH HYPERBILIRUBINEMIA AND PHYSIOLOGICAL JAUNDICE: A LONGITUDINAL STUDY"** is a bonafide work in part of fulfillment for the degree of Master of Science (Audiology) of the student (Register No.A0390009)

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May, 2005

Certificate

This is to certify that this dissertation entitled **"Audiological findings in infants and toddlers with hyperbilirubinemia and physiological jaundice: A longitudinal study"** has been prepared under my supervision and guidance. It is also certified that this has not been submitted earlier in any other University for the award of any diploma or degree.

Guide

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Declaration

This dissertation entitled "Audiological findings in infants and toddlers with hyperbilirubinemia and physiological jaundice: A longitudinal study" is the result of my own study under the guidance of Mr. **ANIMESH BARMAN ,** Lecturer in Audiology, Department of Audiology, All India Institute of Speech and Hearing, Mysore and not been submitted in any other University for the award of any degree or diploma.

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INTRODUCTION

Hearing loss in children is a silent, hidden disability. It is hidden because children, especially infants and toddlers, cannot tell us about their inability to hear well. Hearing is critical for normal speech and language development, which in turn is vital for most aspects of normal human development. A significant hearing impairment at birth can produce a major disruption in language learning (Menyuk, 1977) and produce irreversible deficits in the development of central auditory pathw ays (Moore, 1985). Even a mild hearing impairment has been implicated in delayed development of auditory skills (Quigley, 1978). Early identification of hearing loss followed by appropriate management minimizes the auditory deprivation which can interfere with speech and language learning and central nervous system maturation. It must be stressed that early identification is of little or no value if there are not appropriate follow up measures. As the goal is to detect all the infants with hearing loss, universal hearing screening has been recommended. Several measures that have been recommended for hearing screening include ABR, OAE, immittance, high risk registers.

The causes of hearing loss in infants and toddlers can be many. A few of them, as mentioned by Diefendorf (2002) are, family history of hearing loss, hyperbilirubinemia requiring exchange transfusion, congenital infections (TORCH), craniofacial anomalies, low birth weight, bacterial meningitis, APGAR score of < 3 at 5 minutes, ototoxic medications including aminoglycosides used for more than five days and loop diuretics used in combination with aminoglycosides, prolonged mechanical ventilation for ten days or more and stigmata or other findings associated with a syndrome known to include

sensorineural and/ or conductive hearing loss. Infants, having these risk factors, should be evaluated with a multiple audiometric test battery

Hyperbilirubinemia is one of the most common conditions observed in newborn nursery. The sequaele of hyperbilirubinemia include hearing loss as well as other central nervous system deficits, collectively termed as bilirubin encephalopathy (Evans & Polani, 1950). Bilirubin is a toxic, lipophilic (fat soluble) agent that may alter cellular metabolism by affecting mitochondrial function in the cochlear nuclei and basal ganglia. Increased levels may arise from overproduction of bilirubin combined with decreased clearance of that product of metabolism. In addition, decreased conjugation of bilirubin due to hepatic immaturity may lead to increased levels of relatively fat soluble (and thus able to penetrate CNS) unconjugated bilirubin (Fisch & Osborn, 1954). Clinicopathological studies in neonates of erythroblastosis fetalis (Dublin,1951; Crabtree & Gerrard, 1950) and in experimental animals (Belal, 1975) have demonstrated pathological lesions to be located in the superior olive, lateral laminiscus, and inferior colliculi which take part in the formation of waves III, IV and V of the auditory brainstem response respectively (Picton, Taylor ,Smith & Edwards, 1986). Studies of auditory brainstem responses in hyperbilirubinemic neonates have found abnormalities in latencies of wave III, IV and V, prolonged brainstem conduction time and sparing of cochlear microphonics (Perlman, Fainmessser, & Sohmer, 1983; Kanga, Kitazumi & Kodama, 1979). Further ABR recordings in children with hearing loss following neonatal hyperbilirubinemia have also confirmed that site of bilirubin toxicity is neural rather than cochlear (Chisin, Perlman & Sohmer, 1979; Rance, Beer, Wesson, Shepherd & Domell et al. 1999).

NEED FOR THE STUDY

1) With invent of clinical conditions like auditory dysynchrony and maturational delay the sensitivity and specificity of ABR results has become questionable. And this calls for the administration of an audiological test battery on infants and toddlers who have high risk factors

2) None of the previous studies have carried out behavioral audiometry to correlate with the results of electrophysiological tests. Also most of the studies have evaluated the hearing status of such infants using ABR only and a very few have used OAE (Oysu, Ulubil, Aslan & Baserer, 2000; Rhee, Park & Jung, 1999; Stein, Tremblay, Pasternak, Banerjee & Lindermann, 1996; Berlin, Bordelon, Hurley, Hood & Parkins, 1997; Rance, Beer, Wesson, Shepherd, Dowell, King et al., 1999; Anurag, 2003). Thus this study was taken up to carry out ABR, OAE and BOA in such population which would enable to arrive at appropriate diagnosis.

3) In hyperbilirubinemia cases many studies have attempted to study changes in ABR before and after exchange transfusion (Perlman, Fainmesser, Sohmer, Tamani, Wax & Pensmer,1983; Nwaesei, Aderde, Bayden & Perlman, 1984; Nakamura, Takada, Shimabuku, Matsuo & Negishi, 1985) and a very few studies have attempted to study the maturation of auditory pathway with progressive age in such population(Agarwal, Shukla, Misra, Kapoor & Malik, 1998; Gupta & Mann, 1998: Rhee, Park & Jung, 1999). Thus, this study was taken up to study maturation of auditory pathway with age in such population in terms of correlating changes in ABR and OAE to physiological changes in auditory sensitivity. Also

there are hardly any studies (Nakamura, Takada, Shimabuku, Matsuo, Matsuo & Negishi, 1985; Funata, Tamai, Shimada, & Nakamura, 1994; Amin, Ahlfors, Orlando, Dalzell et el., 2001) which have made an attempt to find the relationship between the peak bilirubin level/direct bilirubin level on auditory system.

4) Not many studies (except Gupta& Mann, 1998; Akman, Ozek, Kulekci, Turkdogan, Cebeci & Akdas, 2004;Anurag, 2004) had the sub group of the hyperbilirubinemic infants based on level and correlated it to the maturational changes based on results obtained in ABR and OAE recordings. However, these were not longitudinal ones.

5) Lack of experience about suitable protocol to assess hearing in infants can lead to wrong diagnosis of infant's hearing capacity. Also different acquisition and stimulus factors like time window, repetition rate, filter settings etc also adds to uncertainty in identifying the waves and interpretation of test results. And therefore studies are required to use:

- Reduced repetition rate which is not found in most of the studies.
- Filter settings of 30 Hz to 3 KHz.
- A time window longer than 10 ms because ABR wave latencies is more likely to be prolonged.

AIM OF THE STUDY

The present study is taken up with the aim of:

1) The effect of hyperbilirubinemia on auditory system based on the results obtained in ABR, OAE and BOA.

2) To determine is there any relationship between peak bilirubin level and direct bilirubin level with hearing loss.

3) The abnormalities seen in test results are reversible or not, if yes, does bilirubin level has any relation with these reversible conditions.

4) Comparison of auditory status in presence of multiple and single high risk factors.

5) To see importance of ABR and OAE as a diagnostic pointer.

6) To find out the site of lesion in these patients.

REVIEW OF LITERATURE

Hyperbilirubinemia is one of the most common problems encountered in term newborns. It is a complex metabolic disorder, which may result from either too much production of bilirubin or too little clearance of bilirubin by the liver (Maisels, 1994).the normal adult serum bilirubin level is less than 1mg/dl. Adults appear jaundiced when the serum bilirubin level is greater than 2mg/dl, and newborns appear jaundiced when it is greater than 7 mg/dl. Between 25 and 50 % of all term newborns and a higher percentage of premature infants develop clinical jaundice. Also, 6.1 % of well term newborns have a maximum serum bilirubin level over 12.9 mg/dl. A serum bilirubin level over 15 mg/dl is found in 3 % of normal term babies (Hinkes & Cloherty, 1998).

Source of bilirubin

Hinkes and Cloherty (1998) described that bilirubin is derived from the breakdown of heme containing proteins in the reticuloendothelial system. The normal newborn produces 6 to 10 mg of bilirubin per kilogram per day, as opposed to the production of 3 to 4mg per kilogram per day in adult. The major heme containing protein is red blood cell (RBC) hemoglobin. Hemoglobin released from senescent RBCs in the reticuloendothelial system is the source of 75 % of all bilirubin production. One gram of hemoglobin produces 34mg of bilirubin. Accelerated release of hemoglobin from RBCs is the cause of hyperbilirubinemia in isoimmunization (e.g., Rh and ABO incompatibility), erythrocyte biochemical abnormalities (e.g. G6PD and pyruvate kinate deficiencies), abnormal erythrocyte morphology (e.g. hereditary spherocytosis), sequestered blood (e.g. bruising and cephalohematoma) and polycythemia. The other 25% of bilirubin is called

early labeled bilirubin. It is derived from hemoglobin released by ineffective erythropoiesis in the bone marrow, from other heme containing proteins in tissues (e.g. myoglobin, cytochromes, catalase and peroxidase) and free from heme.

Bilirubin metabolism

Hinkes and Cloherty (1998) reported that the heme ring from heme containing proteins is oxidized in reticuloendothelial cells to biliverdin. Biliverdin is then reduced to bilirubin by enzyme biliverdin reductase. Catabolism of 1 mole of hemoglobin produces 1 mole each of CO and bilirubin.

Transport: - Bilirubin is non polar and insoluble in water and is transported to liver cells bound to serum albumin. Bilirubin bound to albumin does not usually enter the central nervous system and is thought to be nontoxic. Displacement of bilirubin from albumin by drugs such as the sulfonamides, or by free fatty acids (FFA) at higher molar ratio of FFA: albumin may increase bilirubin toxicity (Hinkes & Cloherty, 1998).

Uptake: Non polar, fat soluble bilirubin (dissociated from albumin) crosses hepatocyte plasma membrane and is bound mainly to cytoplasmic ligandin for transport to smooth endoplasmic reticulum (Hinkes & Cloherty, 1998).

Conjugation: Unconjugated (indirect) bilirubin (UCB) is converted to water soluble conjugated (direct) bilirubin (CB) in smooth endoplasmic reticulum by Uridine Diphosphate Glucuronyl Transferase (UDPG-T). Inherited deficiencies of conjugating enzyme UDPG-T (Crigler-Najjar syndrome) can cause severe hyperbilirubinemia in

neonates. The unconjugated bilirubin is neurotoxic in infants if it is found at high concentrations (Hinkes and Cloherty, 1998).

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Hyperbilirubinemia in newborns

Neonatal hyperbilirubinemia is one of the important problems responsible for significant morbidity and mortality because of raised bilirubin having the capacity to cross the blood brain barrier causing kernicterus. Established kernicterus can be diagnosed easily clinically and has serious permanent sequaele, but by then it is often too late. The early features of bilirubin encephalopathy are reversible, if treated adequately (Bhandari, Narang, Mann, Raghunathan & Bhakoo, 1993). Jaundice in newborns can be either physiological or pathological depending on the level of bilirubin and associated high risk factors. Physiological jaundice occurs when the serum unconjugated bilirubin of newborn infant rises to over 2 mg/dl in the first week of life. This UCB usually rises in full term infants to a peak of 6-8 mg/dl by 3 days of age and then falls. A rise to 12mg /dl is in the physiological range. In premature infants, the peak may be 10 to 12 mg/dl on the fifth day of life, possibly rising over 15mg/dl without any specific abnormality of bilirubin metabolism. Levels under 2mg /dl may not be seen until one month of age in both full term and premature infants. This normal jaundice is attributed to mechanisms like: increased bilirubin production, increased enterohepatic circulatuion, defective uptake of bilirubin from plasma, defective conjugation and decreased hepatic excretion of bilirubin. Infants with identified risk factors rarely have total serum bilirubin levels above 12 mg/dl. As the number of factors increases, the potential to develop markedly elevated bilirubin levels also increases. Hence, infants with multiple high risk factors may develop

an exaggerated physiological jaundice in which the total bilirubin serum level may rise as high as 17mg/dl (Dennery, Seidman and Stevenson, 2001).

Non physiological or pathological jaundice (hyperbilirubinemia) may not be easy to distinguish from physiological jaundice. The situations which can suggest hyperbilirubinemia and require investigation are: onset of jaundice before 24 hours of age, any elevation of serum bilirubin levels that require phototherapy, a rise in serum bilirubin levels of over 0.5 mg/dl/hour, signs of underlying illness in an infant ,jaundice persisting after eight days in a term infant or after 14 days in a premature infant (Hinkes and Cloherty, 1998), also, a total serum bilirubin level higher than 17 mg/dl in a full term newborn and elevation of the serum conjugated bilirubin to greater than 2 mg/dl or more than 20 % of the total serum bilirubin concentration (Dennery, Seidman & Stevenson, 2001; Melton & Akinbi, 1999).

Bilirubin toxicity

Hinkes and Cloherty (1998), reported that the levels of bilirubin that are toxic to one infant may not be toxic to another, or even to the same infant in different clinical circumstances. Bilirubin entry into the brain occurs as free (unbound) bilirubin or as bilirubin bound to albumin in the presence of disrupted brain barriers. It is estimated that 8.5mg of bilirubin will bind tightly to one gram of albumin, although this binding capacity is less in small and sick prematures. Factors that affect blood brain barrier include hypersmolatity, anoxia and hypercarbia. The barrier itself may be more permeable in premature infants.

Kernicterus is a neurologic syndrome resulting from the deposition of unconjugated bilirubin in brain cells. The risk of hyperbilirubinemia is related to the development of kernicterus (bilirubin encephalopathy) at high indirect serum bilirubin levels. The level of serum bilirubin associated with kernicterus is dependent in part of the etiology of the jaundice. Kernicterus develops at lower bilirubin levels in preterm infants and in the presence of asphyxia or drugs that displace bilirubin from albumin. Bilirubin is transported in the bloodstream bound to albumin, a combination felt to be nontoxic. The high affinity of albumin for bilirubin assumes that little unconjugated bilirubin is free to diffuse into other tissues. A low albumin concentration in the low birth weight neonate would result in more bilirubin precipitate in that newborn. Acidosis may predispose to kernicterus since uptake of bilirubin by the mitochondria increases as pH decreases (Brodersen, 1977). Sepsis may result in significant increases in bilirubin concentration by one of the several mechanisms and lead to an increased risk of kernicterus. Increased levels of bilirubin have been reported in bacteremic infants (Rooney, Hill & Danks, 1971). It has been shown that hypothermia may decrease binding of albumin and bilirubin and lead to increased levels of free or direct bilirubin, thus compounding the effects of acidosis and sepsis (Schiff, Stern & Leduc, 1966). The association and dissociation of bilirubin and albumin may occur rapidly, so detection and measurement of free bilirubin in the newborn may be difficult. Thus, the etiology of kernicterus as a cause of hearing loss may be difficult to prove. However, the neonate of low birth weight with associated hypoxia, acidosis and sepsis is at increased risk for the toxic effects of hyperbilirubinemia (Newton, 1985).

Healthy term neonates with bilirubin levels below 25 to 30 mg/dl show little evidence of adverse neurologic outcome (Gartner et al. 1994, cited in Hinkes and

Cloherty, 1998). Vohr (1990) have reported that hyperbilirubinemia in term infants have been associated with abnormalities in brain stem auditory evoked responses, cry characteristics and non behavioural measures. However, these changes disappear when bilirubin level falls and there are no measurable long-term sequaele. Wilkins (1994) reported in his initial early studies of babies with 1250 to 2500gm birth weight and 28-36 weeks gestational age, that there was no relationship between neurologic damage and bilirubin over 18 to 20 mg/dl. Therefore bilirubin toxicity in low birth weight infants may not be a function of bilirubin levels per se but of their overall clinical status.

Management of hyperbilirubinemia

Before treatment is initiated, the minimum evaluation should include the infant's age and postnatal course, a maternal and gestational history, physical examination of the infant and determination of the total serum bilirubin level and the rate at which it is rising (Bhutani, Johnson & Sivieri, 1999).The treatment options for hyperbilirubinemia include phototherapy and blood exchange transfusion. Phototherapy employs blue wavelengths of light to alter unconjugated bilirubin in the skin. The bilirubin is converted into less toxic water soluble photo isomers that are excreted in the urine without conjugation. With intensive phototherapy, the total serum bilirubin level should decline by 1 to 2 mg/dl within four to six hours. If the total serum bilirubin level remains elevated after intensive phototherapy or if initial bilirubin level meets defined criteria levels based on the infant's age, preparations for exchange transfusion are made (Melton & Akinbi, 1999).The following table summarizes the options for medical management of hyperbilirubinemia in term newborns, as given by Hinkes and Cloherty (1998).

Age of the infant(in hours)	Total serum bilirubin (TSB) level, mg/dl			
	Consider (a) phototherapy	Phototherapy	Exchange transfusion if intensive phototherapy fails (b)	Exchange transfusion and intensive phototherapy
< 24 (c)	----------	----------	----------	----------
25-48	> 12	>15	>20	>25
49-72	>15	>18	>25	>30
>72	>17	>20	>25	>30

Table 1: Depicts the management for hyperbilirubinemia in healthy term newborn

a) Photo therapy at these TSB levels is a clinical option meaning that the intervention is available and may be used on the basis of individual clinical judgment.

b) Intensive phototherapy should produce a decline of TSB of 1 to 2mg/dl within 4-6 hours and the TSB level should continue to fall and remain below the threshold level for exchange transfusion. If this does not occur, it is considered a failure of phototherapy.

c) Term infants who are clinically jaundiced at <24 hours are not considered healthy and require further evaluation.

Anatomical lesions associated with hyperbilirubinemia

Morphological study in the jaundiced Gunn rats have illustrated marked alterations of the mitochondria in cells of cochlear nuclei (Jew and Williams, 1977), one of the frequently involved nuclear masses in kernicterus, and also in Purkinje cells of cerebellar cortex (Schutta, Johnson and Neville, 1970). In addition, large membraneous intracytoplasmic bodies are also found in cerebellum cells in experimental animals and in myelinating cerebellar cells exposed to bilirubin in tissue culture (Silberberg, Johnson and Ritter, 1970). Cadaver studies done by Dublin (1951) on seven infants dying from neonatal jaundice suggested that various levels of the auditory pathways showed neuronal damage and the maximum of the neuronal damage occurred at the level of cochlear nuclei. He also reported that bilirubin encephalopathy also affects superior olive, lateral laminiscus and inferior colliculus. He explains that dorsal cochlear nucleus has rich capillary network and therefore is more susceptible to anoxia which increases permeability to bile pigment or unconjugated bilirubin. Cashore (1990) and Hansen (1993) reported that bilirubin induced toxicity involves changes in energy metabolism, alteration in membrane function, decreased membrane potential, alteration in enzyme function, and inhibition of protein and DNA synthesis.

Hyperbilirubinemia and Hearing loss

Neonatal hyperbilirubinemia is a common problem affecting 5-6 % of newborn babies. Persistence of high levels of conjugated and unconjugated bilirubin for long duration may lead to neurologic sequaele among the survivors (Johnston, Angara, Baumal, Hawke, Johnson, Keet & Wood, 1967). High level of bilirubin is particularly

toxic for auditory pathway and may result in sensorineural hearing loss (Fenwick, 1975; Chisin, Perlman & Sohmer 1979; Paul, Thomas & Singh, 1988; Holmes, Miller & Smith, 1968). In 1982, Joint committee on infant hearing (American Academy of Pediatrics) described neonatal hyperbilirubinemia at level exceeding that for exchange transfusion as the significant high risk factor for hearing impairment in their high risk register.

Brainstem auditory evoked responses (BAER) provide useful information regarding the nuerophysiological status of the VIIIth nerve, the cochlear nucleus, superior olivary nucleus, lateral laminiscus and the inferior colliculus in the brainstem (Jewett, 1970; Buchwald and Huang 1975; Starr and Hamilton, 1976). Posticteric sequelae such as hearing loss, as well as other CNS deficits can be shown by the recordings of auditory brainstem response. Wennenberg, Ahlfors, Bickers et al., (1982), mentioned that ABRs may be a useful tool for the monitoring of early bilirubin toxicity in infants who are at risk for jaundice. Gupta and Mann (1998) did a study to find a correlation between increasing levels of serum bilirubin to ABR results and to find the use of ABR as bilirubin neurotoxicity marker. Brainstem conduction time was found to be prolonged from 5.12+ 0.26 to 5.83+0.14 in neonates with a mean serum bilirubin of 16.6mg/dl, where as found to be additionally prolonged to 6.0+1.0 with an increase in mean serum bilirubin level to 21.98mg/dl. At first retest after 1 month, 33.33% of cases with a mean serum bilirubin level of 19.46mg/dl and 86.00% with a mean serum bilirubin level of 15.97mg/dl showed total recovery. After 6 months, three cases with mean serum bilirubin level of 26.3mg/dl and one case with a mean serum bilirubin level of 17.7mg/dl failed to show any improvement. They concluded by saying that neonates with distortion of normal wave patterns on ABR were found to have poorer prognosis compared with those with delayed inter peak latencies.

Exchange transfusion is one of the treatment options for the newborns with jaundice. It removes the bilirubin from the plasma, and so extravacsular bilirubin equilibrates and binds to the albumin in the exchanged blood. Within half an hour after the exchange transfusion, bilirubin levels return to 60 % of pre exchange levels (Michael, Hinkes and John, 1997). Many researchers in the past have recorded ABRs before and after exchange transfusion. Nwaesei, Aerde, Boyden and Perlman (1984) recorded auditory brainstem response before and after exchange transfusion, in an effort to determine whether hyperbilirubinemia is associated with acute effects on brainstem function of neonates. Nine full term infants with hemolytic disease received exchange transfusion for conventional serum bilirubin concentration indications (mean 22.3+1.4mg/dl). In three infants, absence of ABR waves before the exchange transfusion was followed by appearance of the waves after exchange transfusion. Other significant alterations observed in the group after exchange transfusion were increase of wave amplitudes and reduction of inter peak latencies. They concluded that acute brainstem toxicity appears to occur in a percentage of infants with hyperbilirubinemia at serum levels commonly seen in clinical practice and these changes appear to be rapidly reversible with exchange transfusion

Gupta, Raj and Anand (1990) performed ABR on 25 infants with neonatal hyperbilirubinemia at levels exceeding that for exchange transfusion (20 to 25 mg/dl) at a mean conceptional age of 40.4 +0.6 weeks and also on 20 normal term neonates. On the initial evaluation 12 of the hyperbilirubinemic neonates had elevated ABR thresholds and prolonged wave latencies which improved in all the infants on follow up retesting at three months. Wave V, however, was present at 30dBnHL click stimulus in all the normal

neonates. Therefore, they suggested neonatal jaundice was associated with significant transient aberrations of ABR, suggestive of a transient toxic brainstem encephalopathy.

Bhandari, Narang, Mann, Raghunathan and Bhakoo (1993) studied Auditory evoked responses using BERA in 30 new born babies with plasma bilirubin >15mg/dl and repeated after exchange transfusion with bilirubin levels of <10mg/dl. A few jaundiced babies (16.5%) should absent BERA responses at the initial and subsequent examination. After treatment, 3/30 babies showed absent waveform responses and 2 of these were clinically kernicteric. Jaundiced babies had prolonged wave peak latencies and inter peak latencies. Treated babies showed a tendency towards recovery in their BERA responses which were however not complete. Total plasma bilirubin value at the time of BERA examination and mean maximal bilirubin values had no correlation with the incidence and degree of BERA abnormalities.

Funata, Takada, Tamai and Shimada (1996) examined the changes in ABR in hyperbilirubinemic newborns before and after exchange transfusion, in order to check their usefulness in the early detection of acute and chronic bilirubin encephalopathy. ABR were measured in 10 infants with bilirubin concentration >or = 20mg/dl before and after exchange transfusion (ET). The ABR measurements were performed before ET and 6 days after the ET. The follow up of ABR was performed at 3 months of life. In comparison with the control values, the mean latencies of ABR were significantly prolonged (I, I-III, and I-V) and the mean amplitude were significantly decreased (I, III, and V) before exchange transfusion. Significant improvement of ABR was noticed after the exchange transfusion, especially in the shortening of the latency of wave I and in increasing the amplitude of wave III and V, though the recovery of the latency of I-V and

the amplitude of I, III and V wave were delayed in comparison to the control. The follow up testing of ABR showed that, in two of nine infants there were still abnormal findings at 3 months of age. Only one of these who had prolonged ABR waves, the recovery of ABR was observed until 5 years of age. They suggested that delay in improvement of ABR abnormally might be possibly used as an early warning signal for following chronic bilirubin encephalopathy.

Agarwal, Shukla, Misra, Kapoor and Malik (1998) carried out ABR on 30 term neonates with hyperbilirubinemia (bilirubin level> 15mg/dl) before exc hange transfusion at peak of hyperbilirubinemia, after exchange transfusion and at age of 2-4 months. They also recorded ABR for 25 normal term neonates. 17 out of 30 neonates with hyperbilirubinemia showed abnormalities on initial ABR recording. The most common abnormality seen was elevated threshold of wave V in 12 neonates. Other abnormalities observed were absence of all waves at 90dB (23.30%), prolongation of latencies of all waves (26.70%) and prolongations of various intervals (26.70%). Abnormalities in ABR correlated significantly with bilirubin level. After treatment abnormalities reversed back to normal in 10 cases but persisted in 7 out of 17 (41.17%) cases with initial abnormal ABR. 3 infants out of these had persistent abnormalities in ABR. Thus they suggested that ABR abnormalities can be transient or chronic which significantly correlates with level of serum bilirubin and duration of jaundice.

The above mentioned studies have demonstrated an improvement in ABR responses after exchange transfusion in majority of the cases. These improvements are considered to have been brought about by removal of bilirubin from the body and from the brainstem during exchange transfusion.

Serum total bilirubin levels remains primary biochemical measure used to evaluate and treat premature newborns with hyperbilirubinemia, although there is substantial evidence that total serum bilirubin levels correlate poorly with bilirubin induced neurotoxicity (Silverman, Andersen, Blanc & Corizer, 1956; Kim, Yoon, Sher & Brown, 1980; Turkel, Guttenberg, Moynes & Hodgman, 1980; Lucey, 1982; O'Shea, Dillard, Klinepeter & Goldstein, 1992). A very few studies have tried to assess the relationship between direct bilirubin levels and hearing loss in infants. Nakamura, Takada, Shimabuku, Matsuo, Matsuo and Negishi (1985) studied the relationship between ABR and total bilirubin levels as well as unbound bilirubin levels in 56 hyperbilirubinemic infants and 24 infants who did not have jaundice. The latencies of wave I at 85dBnHL in hyperbilirubinemic infants were significantly greater than those in the control group. The latencies of wave I and V in hyperbilirubinemic infants with unbound bilirubin levels = 1.0mg/dl were greater than those in the control group and in the hyperbilirubinemic infants with unbound bilirubin < 0.5mg/dl and with unbound bilirubin levels < 1.0mg/dl. The ABR wave latencies were noticed at 24, 48 and 96 hours after exchange transfusion which were prolonged before the transfusion but reduced at 48 and 96 hours after the exchange transfusion. Improvement of wave V suggests that auditory nerve is reversibly damaged in infants with hyperbilirubinemia and abnormal ABR are more closely related to the unbound bilirubin level than the total bilirubin level.

Funata, Tamia, Shimada and Nakamura (1994) measured ABR and serum unbound bilirubin concentration (UBC) in 37 hyperbilirubinemic term newborns with total bilirubin concentration (TBC) >20mg/dl and direct bilirubin concentration <2mg/dl before treatment with either phototherapy or exchange transfusions. These hyperbilirubinemic new borns were divided into three groups according to the findings of

ABR: group A, normal ABR (n=18); group B, prolonged latency of wave, only (n=8); and group C, prolonged inter peak latency of wave I-III/I-V and/or poor amplitude (n=11). The peak total bilirubin concentrations was significantly different between group A and C (22.8+2.2mg/dl and 25.4+2.5mg/dl) though there were no differences between groups A and B and between groups B and C. The peak UBC in groups B (1.27+ .7 micro g/dl) and C (1.34+ .37 microgram/dl) were significantly higher than in group A (.78+ .26 microgram/dl). Though there were no significant differences in the peak UBC between groups B and C abnormal ABR findings were more clearly associated with the level of UBC at 1.0mg/dl than that of TBC at 23 mg/dl. These results suggest that measuring UBC may help in evaluating the possible risk of bilirubin encephalopathy in full term newborns where there is vigintiphobia.

Amin, Ahlfors, Orlando, Dalzell et al., (2001) evaluated 143 infants using ABR over a two year period. They found that the maturation of the ABR responses in the hyperbilirubinemic infants did not correlate with the peak serum bilirubin levels. However, in infants the mean peak unbound was significantly higher in infants with abnormal maturation (n=20) than in infants with normal maturation (n=20). Hence, they suggested that unbound bilirubin level is a more sensitive predictor of hearing loss as compared to total bilirubin level.

Role of multiple risk factors in producing hearing impairment is not highlighted in the high risk registers provided by Joint Committee on Infant Hearing. However, presence of multiple high risk factors can lead to a higher risk of hearing loss as compared to those with single risk factors. A study was carried out by DeVries, Larry and Dubowitz (1985) on 99 infants having total bilirubin level > 14mg/dl who were divided into high risk or

low risk infants on the basis of perinatal risk factors. Eight of the twenty two high risk infants with birth weight < 1500gm but only two of forty three high risk with birth weight >1500gm were deaf. They suggested that in healthy preterm infants with birth weight >1500gm, high bilirubin levels carry little risk, where as serum bilirubin level > 14mg/dl in high risk preterm infants with birth weight 1500gm or less is associated with a high risk of deafness.

Gupta, Anand and Raj (1991) recorded ABR for 68 neonates having one or more adverse perinatal clinical factors viz prematurity (<37 weeks), low birth weight (<2000gm), hyperbilirubinemia requiring active intervention, birth anoxia, neonatal seizures and infections. ABR was performed within first 6 weeks of life at a mean conceptional age of 40.2+0.6 weeks. 13 of 68 neonates were found to have hearing impairment. Elevated auditory threshold was found more frequently in neonates with multiple clinical adverse factors than those having single risk factor (6/13 vs. 7/55, P<0.001). On multiple logistic regression analysis, however, indicated that, only 2 factors viz: hyperbilirubinemia at level exceeding indication for exchange transfusion and birth weight <1500gm were found to be significantly correlated with hearing impairment. Similar observations are also noticed by Mjoen, Langslet, Tangsrud and Sundby (1982) and Galambos, Hicks and Wison (1982).

Several investigators suggested that hyperbilirubinemia could also be a cause auditory neuropathy. Chisin, Perlman and Sohmer (1979) were among the first to report that, in patients with no neural response from the auditory nerve and brainstem by ABR following neonatal hyperbilirubinemia, there was evidence for damage to the auditory nerve while the cochlea was spared. Since then several investigators have tried to see the

relationship between hyperbilirubinemia and auditory dysynchrony. Stein, Tremblay, Pasternak, Banerjee, Lindermann and Kraus (1996) observed in their screening program infants who failed hearing screening on ABR, but passed their OAE test. Four out of five of them had hyperbilirubinemia. Rance, Beer, Wesson, Shepherd, Dowell, King, et al., (1999) reported that auditory neuropathy occurred in 10 of 20 patients with bilirubin concentrations greater than 20 mg/dl. Rhee, Park and Jung (1999) did an audiological evaluation of neonates with severe hyperbilirubinemia using TEOAE and ABR. Out of eleven neonates four had abnormal or no ABR response and the other seven demonstrated normal response in ABR. All 11 neonates passed TEOAEs. However, two neonates showed improved in auditory function at 3 or 6 months follow up ABR.

Oysu, Ulubil, Aslan and Baserer (2000) reported that neonatal hyperbilirubinemia is an important cause of childhood deafness, especially in developing countries. After neonatal hyperbilirubinemia, the auditory neural pathways, cochlea, or both may be affected. They assessed 1,032 pediatric patients with hearing loss revealed 67 cases of serve hyperbilirubinemia as the single identifiable risk factor for hearing loss. In 26 of 30 cases, OAEs were absent whereas in the remaining four cases, robust emissions were detected despite an absent ABR. They recommended dual screening of hearing by ABR and OAEs in hyperbilirubinemic newborns.

Akman, Ozek, Kulekci, Cebeci and Akdas (2004), tried to evaluate whether a correlation exists between increased serum bilirubin and Neuron Specific Enolase (NSE) assays and auditory neuropathy. They recorded auditory evoked responses and transient evoked otoacoustic emissions (TEOAEs) for nineteen term neonates without hemolysis whose serum bilirubin levels were above 20 mg/dl and twenty seven healthy term

newborns with bilirubin levels < 13 mg/dl. All the infants were found to have normal TEOAEs but seven of nineteen infants showed no repeatable ABRs bilaterally to alternating click polarity at the maximum intensity level. Of these seven infants with auditory neuropathy, six infants were from group B (mean peak serum bilirubin level from 25.2 to 40.8 mg/dl) and only one was from group A (mean peak serum bilirubin level from 20.0 to 23.7 mg/dl). They could not demonstrate any correlation between the serum NSE and bilirubin values. However, infants who had auditory neuropathy had significantly higher NSE levels, and thus they recommended that the subjects in this high risk group need close follow up.

Anurag (2003) carried out single recordings of ABR and DPOAE in 15 infants who were divided into four groups depending on the results obtained on ABR and DPOAEs. Group I consisted of seven infants with bilirubin levels < 15 mg/dl. All the infants in this group showed ABR responses till 30 dBnHL and normal DPOAEs. Group II included 5 infants with auditory neuropathy with bilirubin levels > 20 mg/dl. Infants (n=2) in group III had ABR and DPOAEs absent and had bilirubin levels between 16-20 mg/dl. One infant in group IV with bilirubin level 27 mg/dl demonstrated presence of ABR in one ear and absent in one ear whereas DPOAEs present in both the ears. However, there is no one to one correlation between bilirubin level and ABR and OAE findings.

Hence, the results of these studies suggest that the main site of lesion in majority of the cases, if not all, with hearing loss caused by hyperbilirubinemia may be at the retrocochlear location while the cochlea remaining intact.

Thus, the above review suggests a systematic study of ABR and OAE recording along with behavioral observation audiometry (BOA), is required in infants with hyperbilirubinemia. It also suggests one must consider both peak and direct bilirubin level to find the relationship between hyperbilirubinemia and its effect on auditory system. A few investigations do indicate the necessity of successive follow up of those infants with absent or abnormal ABR and presence of OAE and behavioral responses observed at any given level with hyperbilirubinemia. Thus, having all this in mind, this study has been taken up to see the relationship between peak serum bilirubin/ direct bilirubin level and hearing loss and also to observe is there any chance of delayed auditory maturation which might lead to reversible audiological findings.

METHOD

To accomplish the aim, the following method was planned.

Subject selection criteria

Fourteen subjects were considered for the present study whose first evaluation was completed within one year of birth. The subjects had a history of physiological jaundice or hyperbilirubinemia during their neonatal period. These subjects were then divided into three groups depending on their peak serum bilirubin level, details of which are depicted in tables 3, 5 and 7 in following section.

Group A: four subjects with bilirubin level < 15 mg/dl.

Group B: five subjects with bilirubin level 16 – 20 mg/dl.

Group C: five subjects with bilirubin level > 20 mg/dl.

All the audiological assessments for all the subjects taken for the study were carried out after the treatment for hyperbilirubinemia i.e. phototherapy or exchange transfusion.

Instrumentation

1. A calibrated two channel diagnostic audiometer OB922 with impedance matched speakers were used to obtain behavioral responses.

2. TEOAEs were acquired employing ILO292 (software version 5) in TE Full menu option, in order to examine the status of the outer hair cells.

3. Nicolet Bravo, auditory evoked potential system version 3.0, was utilized to record Auditory Brainstem Responses (ABR).

4. An immitance meter Grason Stadler Inc. (GSI) Tympstar was used to run tympanometry in cases with absent OAEs to rule out middle ear pathology.

Calibration of all the instruments was ensured prior to use as recommended by the manufacturer.

Test Environment

All tests were carried out in a well illuminated air conditioned room which was acoustically treated and had ambient noise levels within the permissible limits as recommended by ANSI 1989 (as cited in Mascarenhas,2001).

Test procedure

Detailed information regarding the history of the prenatal, natal and postnatal medical conditions was secured for all the subjects. Medical reports regarding this were reviewed to make a note of various risk factors, if any, and other associated medical conditions. An effort was made to mark the severity of the risk factors mentioned in the reports. A detailed report regarding the auditory behavior of the child at home for various

environmental sounds like calling bell, dog bark, voices from a radio or television pressure cooker whistle, noise made by a grinding machine, water falling into a bucket, name call etc were obtained from the parents. The parents were counseled regarding the importance of follow up and were instructed to observe the auditory behavior of their child at home and report of changes, if any, during a brief interview with the clinician in the follow up evaluation. A total of three evaluations were conducted for each subject with a time interval of approximately three months between two successive evaluations.

Test Battery

The test battery consisted of the following:

1) Behavioral Observation Audiometry: The behavioral responses of the child were observed in free field condition using warble tones of 500Hz, 1 kHz, 2 kHz and speech stimuli. It was carried out in double room situation. The subjects were seated comfortably on the caregiver's lap at a distance of 1 meter from both the speakers at an azimuth of 45 degrees, in the observation room. One clinician was present in the observation room to draw the attention of the child to the midline and to watch for the unconditioned responses. The other clinician, in the test room, presented the test stimuli sequentially with the initiation level being decided below the level at which the child expected to exhibit some kind of an auditory behavior, as reported by the parents. The lowest level at which behavioral responses were exhibited by the subject for each stimulus was noted.

3) Auditory Brainstem Response: ABR was recorded when the child was asleep. Electrode placement: the electrode sites were cleaned using skin preparing paste.

Adequate amount of conduction material and a piece of plaster were used to fix the silver chloride disc type electrodes. The non inverting electrode was placed on the high forehead (Fz) and the inverting electrode on the test ear's mastoid (A1/A2) with the common being placed on the non test ear's mastoid (A2/A1). The independent electrode impedance and inter electrode impedance was maintained well within 5 KOhms. Head set with ear phones (blue on left and red on right ear) was placed taking care not to dislodge the electrodes. Placement of earphones was such that the earphone diaphragm was in alignment with the opening of the ear canal, so that accurate stimulus intensity levels were delivered to the ear. TDH-39 P headphones encased in TELEX-C03624 ear cushions were used for the same. The parameters used to record ABR were:

General setup	Amplifier setup	Channel	Stimulus parameters
Test: AEP Number of channels:1	Sensitivity: Band pass filter: Notch filter: Artifact rejection: Time window:	50 micro V Low pass filter: 3kHz High pass filter: 30Hz Off On 15 ms (less mature auditory system of infants may display wave characteristics beyond the conventional window size of 10ms)	Type: clicks Polarity: rarefaction Intensity: variable Number of stimuli: 1500 Repetition rate: 11.1/s (was adopted because the infant auditory system which is poorly established will flaunt a better morphology with a lower repetition rate).

Table 2: Depicts the amplifier settings and stimulus parameters used for ABR recording.

The level of testing was decided depending on the BOA result. Infants or toddlers having BOA responses at higher levels were tested at 90dBnHL, and the intensity was reduced by 20 or 10 dB step, depending on the wave morphology, till no observable Wave V could be detected. Intensity was increased by 10dB to estimate the threshold whenever it was required. Each recording was duplicated at the threshold or near threshold level to confirm the presence of wave V. The absolute latency at each intensity was noted for the subjects for whom the ABR responses were present.

4) **Otoacoustic Emissions**: TEOAEs were obtained with the foam tip of the probe positioned in the external auditory canal so as to give a flat stimulus spectrum across the frequency range. The filter setting of the stimuli was from 500 Hz to 6000 Hz. 80 microseconds rectangular pulses (clicks) presented at 20 msec intervals and at an intensity of approximately 80dBpSPL in the ear canal. Repetitions about every 20 msec and synchronous averaging allow the signal to noise ratio of the complex OAE waveform to be enhanced as required. A total of 260 averages, above the automatic noise rejection level of the instrument were stored for analysis. The presentation mode included a series of four stimuli, three stimuli at the same level and polarity and a stimulus three times greater in level and inverted in polarity. This was done so as to minimize the artifacts. A response was considered an emission depending on its reproducibility and signal to noise ratio. The emission had to be reproducible atleast 50% of the time (Kemp 1990) and have an S/N ratio of >/= +3 dB (Dijk and Wit, 1987) for it to be considered as a presence of an echo or emission.

5. **Immitance:** Whenever absent echoes were encountered, general tympanometric measures were administered with the probe tone being 678 Hz. This was to ensure

that absence of OAEs were not absent due to the presence of middle ear pathology. Appropriate probe tips were used to obtain a proper seal at a comfortable pressure for the subject. The parameters documented were the type of the tympanogram, the ear canal volume, the static compliance and the tympanometric peak pressure. Middle ear effusion was indexed positive whenever the values were deviant from the norms. The results were later correlated with the ENT findings for confirmation.

Analyses

The results of behavioral observation audiometry, auditory brainstem response and otoacoustic emissions were tabulated for the three different groups separately which were further interpreted using descriptive analyses.

RESULTS AND DISCUSSION

This study was taken up with the aim of studying the following:

1) The effect of hyperbilirubinemia on auditory system based on the results obtained in ABR, OAE and BOA.
2) To determine if there is any relationship between peak bilirubin level and direct bilirubin level with hearing loss.
3) The abnormalities seen in test results are reversible or not, if yes, does bilirubin level has any relation with these reversible conditions.
4) Comparison of auditory status in presence of multiple and single high risk factors.
5) To see importance of ABR, OAE and BOA as a diagnostic pointer.
6) To find out the site of lesion in these patients.

To accomplish the above mentioned aims the fourteen subjects were taken for the study. They were again divided into three groups depending on their peak bilirubin level. Group A (< 15 mg/dl) consisted of four subjects and group B (16 to 20 mg/dl) and C (>20 mg/dl) both had five subjects each. The bilirubin level (direct, indirect and peak), other associated high risk factors, auditory behavior of the infant observed by parents at home and the treatment given for high levels of bilirubin are summarized in the table 3, 5 and 7 and the ABR, OAE and BOA results of the subjects for all the three evaluations are summarized in table 4, 6 and 8 for group A, B and C respectively.

SUBJECT CHARACTERSTICS OF GROUP A: PEAK BILIRUBIN LEVEL < 15 mg/dl

Subject	Age of first Evaluation / Sex	Bilirubin Level	Additional Risk Factors	Auditory Behavior as reported by parents	Mode of Treatment
1	5m/M	14 mg/dl Direct-0.55 mg/dl Indirect-13.45 mg/dl	Premature by 6 weeks Sepsis Kept in incubator Low birth weight	Responds to soft auditory stimuli	Phototherapy
2	5m/F	11.1 mg/dl Direct-0.50 mg/dl Indirect-10.60 mg/dl	Premature by 6 weeks Kept in incubator Low birth weight Congenital heart disease	Responds to soft auditory stimuli	Phototherapy
3	5m/M	14.3 mg/dl Direct-1.3 mg/dl Indirect-12.93 mg/dl	Premature baby Very Low birth weight	Responds to very loud auditory stimuli	Phototherapy
4	6m/F	8.3 mg /dl Direct-1.0 mg/dl Indirect-7.3 mg/dl	Elder brother reported to have hearing loss	Responses are not observed for different auditory stimuli even for loud stimulus.	Phototherapy

TABLE 3: Depicts the detailed case history of each case in group A.

Case	No.of Evaln	ABR Latencies (msec)			Intensity (dBnHL)	ABR Latencies (msec)			OAE		BOA (responses in dBHL)			
		Right Ear				Left Ear								
		I	III	V		I	III	V	Right Ear	Left Ear	500 Hz	1 K Hz	2 K Hz	Speech
1	I	-	4.13	6.44	70	-	-	6.12	Present	Present	40	40	45	35
		-	5.20	6.96	50	-	-	7.40						
		-	5.56	7.60	30	-	-	7.41						
	II	N.A.			50	-	4.58	7.08	Present	Present	35	35	30	30
		-	5.64	7.68	30	-	5.22	7.42						
	III	-	-	7.45	30	-	-	7.30	Present	Present	30	35	30	30
2	I	-	4.65	6.66	50	-	4.76	6.82	Present	Present	40	45	35	40
		-	5.22	7.44	30	-	5.34	7.62						
	II	-	4.92	6.66	50	N.A.			Present	Present	35	35	30	35
		-	5.72	7.44	30	-	-	7.52						
	III	-	-	7.30	30	-	-	7.25	Present	Present	30	30	30	30
3	I	N.R.			90	N.R.			Absent	Absent	N	NR	NR	NR
	II	N.R.			90	N.R.			Absent	Absent	NR	NR	NR	95
	III	N.R.			90	N.R.			Absent	Absent	NR	NR	NR	90
4	I	N.R.			90	N.R.			Absent	Absent	NR	NR	NR	NR
	II	N.R.			90	N.R.			Absent	Absent	NR	NR	NR	NR
	III	N.R.			90	N.R.			Absent	Absent	NR	NR	NR	NR

TABLE 4: Depicts the ABR, OAE and BOA findings of each subject during each sitting for group A.

*N.A. - Not administered
*N.R. - No response

This group consisted of four neonates (two males and two females), in whom neonatal physiological jaundice was observed during first six days of life and the serum bilirubin concentrations measured were less than 15 mg/dl. As can be seen in table 3, subject 1 and 2 were premature and had low birth weight. They had other associated risk factors also which is mentioned in table 3. Subject 3 was a premature and had very low birth weight and subject 4 did not have any associated risk factor except a family history of hearing loss.

It is evident from table 4 that, auditory brainstem response could be recorded bilaterally at 30 dBnHL in subject 1 and 2. TEOAEs were also present in both the ears in all the three successive evaluations. The behavioral responses did correlate well with ABR and OAE results. In subjects 3 and 4, ABR waves could not be identified even at 90 dBnHL in three successive evaluations done at three and six months later. No observable emissions could also be recorded in all the three evaluations. Tympanometry results did not reveal any middle ear pathology. Behavioral responses could not be observed at highest audiometric levels. All these results suggest that subject 3 and 4 have severe hearing loss in both the ears.

Subject 1 and 2, though had other high risk factors associated to physiological jaundice, as can be seen in table 3, had normal outer hair cell functioning and auditory nerve, as normal audiological findings could be obtained here. A similar observation was also noticed by Anurag (2003) in seven infants with serum bilirubin level < 15 mg/dl having normal results on ABR and OAE results. De Vries, Lary and Dubowitz (1985), also observed that in healthy preterm infants with birth weight greater than 1500 grams , high bilirubin levels carry little risk of deafness, whereas a serum bilirubin level greater than 14 mg/dl in

high risk infants with a birth weight of 1500 gm or less is associated with a high risk of deafness.

Gartner et al. (1994, cited in Hinkes and Cloherty, 1998) reported that bilirubin levels below 25 – 30 mg/dl show little evidence of adverse neurologic outcome in healthy term neonates. Akman et al. (2003), suggested that one should be concerned by bilirubin levels greater than 20 mg/dl in healthy term neonates. Therefore bilirubin levels less than 15 mg/dl should be safe for healthy term neonates. Whereas this level of bilirubin in infants with multiple risk factors may give rise to exaggerated physiological jaundice which might cause some forms of damage to the auditory system (Dennery, Seidman and Stevenson, 2001). It can be noticed that, subject 3 had severe hearing loss with a total bilirubin level of 14.3 mg/dl. The problem of neurotoxicity in this subject could be exacerbated due to the presence of other high risk factors as mentioned in the table 3 (very low birth weight and prematurity). Various clinical factors, such as hypothermia, hypoxia, acidosis, hypercarbia, asphyxia, sepsis, severe intraventricular hemorrhage, and hemolysis have been postulated to explain the occurrence of bilirubin neurotoxicity at much lower levels of serum total bilirubin (Amin et al. 2001). In term healthy neonates binding properties are fairly constant (Wennenberg, 1992) but in neonates with multiple risk factors bilirubin binding to albumin may be affected by presence of competitors for bilirubin binding and therefore resulting in a higher direct bilirubin level. Hence, suggesting toxicity may not be a function of bilirubin levels per se but of overall clinical status. Thus, the overall clinical status rendered the auditory system to be more susceptible and therefore resulting into permanent damage. Gupta, Anand and Raj (1991) suggested that elevated auditory threshold was found more frequently in neonates

with multiple clinical adverse factors than in those having single clinical factor. Similar reports have also been given by Mjoen, Langslit, Tangrred and Sindby (1982) and Galambos and Despland (1980). It can be noticed from table 4 that in subject 3 and 4 the functioning of both outer hair cells and auditory nerve is affected. It is possible that outer hair cells of these children have undergone sufficient insult to disrupt the active process due to bilirubin induced toxicity which involves changes in energy metabolism, alteration in membrane function, decreased membrane potential, alteration in enzyme function, and inhibition of protein and DNA synthesis (Cashore, 1990; Hansen, 1993). Similar kind of toxic changes might have occurred in outer hair cells of these two subjects with absent OAEs which probably disrupted the motile aspects of outer hair cell function resulting in absent OAEs.

The level of bilirubin for subject 4 is 8.3mg/dl which is not actually toxic to the auditory system and neither this child was a high risk baby. The presence of hearing loss in this subject is probably due to a genetic cause as the infant's elder brother is also reported to have bilateral severe hearing loss which was reported to be idiopathic.

Another possible cause for subject 3 and 4 having hearing loss could be due to the direct bilirubin level which was > 1mg/dl rather than peak bilirubin level. This measure is more clearly associated with ABR abnormalities, as reported by Funato et al. (1994) and Nakamura et al (1985). They reported that abnormal ABR finding, such as prolonged ABR wave latencies and absence of ABR waves is more clearly associated with the direct bilirubin level = 1 mg/dl rather than total bilirubin level. Funato et al. (1994) have reported that in term neonates' unbound or direct bilirubin levels must reach to 1-2 mg/dl before bilirubin

induced ABR changes are likely. But in premature infants, ABR changes appear as the unbound concentrations exceed 0.5mg/dl (Nakamura, Yonetani, Vetani, Funato & Lee, 1992; Cashore, 1982; Ritter, Kennery, Norton & Rudoph, 1982). The pathogenesis of bilirubin encephalopathy is complex. Clinical factors, bilirubin albumin binding, and the integrity of the blood brain barrier are all thought to play significant roles in bilirubin toxicity. In term neonates, direct bilirubin has been reported to be a more sensitive predictor of bilirubin encephalopathy as evaluated by ABR than total serum bilirubin level (Nakamura et al. 1985). Current data support the notion that unbound bilirubin is capable of crossing the intact blood brain barrier and causing subsequent neuronal damage especially when it crosses 1 mg/dl. Probably, the presence of very low birth weight and prematurity, in subject 3, affected the serum bilirubin–albumin binding, bilirubin entry in the brain, or tissue uptake of bilirubin and thus causing bilirubin toxicity to occur at a lower serum bilirubin levels. This also explains the difference in auditory status of subjects 1 and 3, who having similar total bilirubin level of 14 mg/dl and 14.3 mg/dl respectively, had different direct bilirubin level, which is 0.55 mg/dl and 1.3mg/dl respectively.

The current result indicates that direct bilirubin level is a better correlate of determining damage to the auditory system as compared to the total bilirubin level. A direct bilirubin level > 1mg/dl is more significant, in terms of toxicity to the auditory system as compared to a direct bilirubin level < 1 mg/dl. In infants with multiple high risk factors, the bilirubin binding properties are altered leading to a higher direct bilirubin level which might in turn results bilirubin toxicity at lower total serum bilirubin levels. Therefore, peak serum bilirubin levels below 15 mg/dl might lead to an insult to auditory system only when it's

associated with direct bilirubin level > 1mg/dl and/or associated with other high risk factors especially very low birth weight.

SUBJECT CHARACTERSTICS OF GROUP B: PEAK BILIRUBIN LEVEL 16 – 20 mg/dl

Subject	Age of first Evaluation / Sex	Peak Bilirubin Level	Additional Risk Factors	Auditory Behavior as observed by parents	Mode of Treatment
1	9 m/F	16.1 mg/dl Direct- 0.7 mg/dl Indirect-15.4 mg/dl	Premature baby	Responds to moderately loud auditory stimuli	Blood transfusion
2	3 ½ m/F	16 mg/dl Direct-1.12mg/dl Indirect-14.88 mg/dl	Low birth weight Septicemia	Responds to loud auditory stimuli	Phototherapy
3	9 m/F	17.3 mg/dl Direct-1.62 mg/dl Indirect-15.68 mg/dl	Premature baby Delayed birth cry Hypoxia One pair of twin died	Does not respond to loud auditory stimuli	Blood transfusion
4	6m/F	17.2 mg/dl Direct-1.51 mg/dl Indirect-15.69mg/dl	Premature baby Low birth weight	Responds to loud auditory stimuli	Phototherapy
5	1 Y / F	19.8 mg/dl Direct-1.5 mg/dl Indirect- 18.3 mg/dl	Premature baby Kept in incubator Kernicterus	Responds to loud auditory stimuli	Blood transfusion

TABLE 5: Depicts the detailed case history of each case in gropu B.

Case	No. of Evaln.	ABR Latencies (msec)			Intensity (dBnHL)	ABR Latencies (msec)			OAE		BOA (responses in dBHL)			
		Right Ear				Left Ear								
		I	III	V		I	III	V	Right Ear	Left Ear	500Hz	1KHz	2KHz	Speech
!	I	-	-	6.1	90	N.A.			Present	Present	50	55	55	45
		N.A.			80	-	-	6.0						
		N.R.			70	-	-	-						
		N.A			60	-	-	7.0						
		N.A.			50	N.R.								
	II	1.6	4.0	5.8	90	N.A.			Present	Present	40	50	45	40
		N.A.			70	-	-	8.5						
		-	-	6.7	50	-	-	9.5						
		N.A.			40	-	-	10						
		-	-	8.2	30	N.R.								
	III	N.A.			70	1.8	3.8	5.5	Present	Present	30	30	30	30
		4.3	7.1	8.7	50	2.1	4.5	6.2						
		-	-	9.2	40	N.A.								
		-	-	9.3	30	-	-	7.9						
2	I	N.R.			90	N.R.			Absent	Absent	N.R.	N.R.	N.R.	N.R.
	II	N.R			90	N.R.			Absent	Absent	N.R.	N.R.	N.R.	N.R.
	III	N.R.			90	N.R			Absent	Absent	N.R.	N.R.	N.R.	N.R.
3	I	N.R.			90	N.R			Absent	Absent	N.R.	N.R.	N.R.	N.R.
	II	N.R.			90	N.R.			Absent	Absent	N.R.	N.R.	N.R.	N.R.
	III	N.R.			90	N.R.			Absent	Absent	N.R.	N.R.	N.R.	N.R.
4	I	N.R.			90	N.R.			Absent	Absent	N.R.	N.R.	N.R.	N.R.
	II	N.R.			90	N.R.			Absent	Absent	N.R.	N.R.	N.R.	N.R.
	III	N.R.			90	N.R.			Absent	Absent	N.R.	N.R.	N.R.	N.R

5	I	N.R.	90	N.R.	Present	Present	95	90	90	90
	II	N.R.	90	N.R.	Present	Present	90	85	85	80
	III	N.R.	90	N.R.	Present	present	85	85	85	80

TABLE 6: Depicts the ABR, OAE and BOA findings of each subject during each sitting for group B.

N.A. - Not administered
N.R - No response

This group consisted of five subjects with bilirubin levels between 16 – 20 mg/dl. Table 5 depicts the associated risk factors and the bilirubin levels of each case in group B. All the subjects were premature babies except subject 2. Two of them i.e. subject 2 and 4, had low birth weight.

It can be seen in table 6, three out of five subjects (2, 3 and 4) had absent OAEs and ABRs on all the three evaluations. Tympanometry results revealed that the absence of OAEs was not due to the middle ear pathology. Subject 5, had robust OAEs but no ABR at initial evaluation. On two successive assessments, ABR could not be detected, confirming that the child is likely to have irreversible auditory dysynchrony. Behavioral responses for this child could be observed at higher audiometric levels as mentioned in table 6. Case 1 has a different clinical entity, who had OAEs present on all the evaluations whereas ABR morphology and threshold have improved with repeated recordings over time. For right ear no visually detectable ABR waves could be depicted, except perhaps a fragment of wave V in the initial recording. Wave V responses were very apparent in the left ear and replicated down to 60 dBnHL. After three months, ABR demonstrated a visible I, III and V at 90 dBnHL in right ear with ABR threshold being 30 dBnHL. On left ear, however, only wave V continued to be observed till 40 dBnHL. In the third follow up assessment, wave I, III and V could be detected at 50 dBnHL and wave V could be recorded till 30 dBnHL, bilaterally. Therefore, this case showed an overall improvement of 60 dB in right ear and 20 dB in left ear from first to third recording. A subsequent improvement in BOA results was also observed in this subject.

Hearing deficit used to be a relatively common sequaele of bilirubin encephalopathy (Crabtree & Gerrard, 1950), possibly owing to brainstem lesions (Nwaesei et al., 1984). However, with the current management of neonatal hyperbilirubinemia the incidence of bilirubin encephalopathy is probably decreasing (DeVries, Lary & Dubowitz1987). Nevertheless, some researchers have detected subtle damage to brainstem in neonates with hyperbilirubinemia at relatively lower levels (Perlman et al., 1983). Three cases in this group demonstrated a permanent irreversible damage to auditory system which was elucidated by absence of ABR on the successive follow up audiological examinations. These three subjects had overall bilirubin levels which can be toxic to infant's central nervous system especially in high risk babies. Also the three infants had other high risk factors which could have altered the bilirubin binding properties and tissue uptake of the bilirubin as discussed for subject 3 in group A rendering bilirubin to be more toxic to the auditory system.

Subject 5 demonstrated the presence of normal functioning of outer hair cells but indicating that high levels of bilirubin can have a differential effect to the auditory nerve and the cochlear hair cells which is reflected in ABR being absent and presence of OAEs in all the three evaluations. The idea of cochlear sparing in hyperbilirubinemia was first proposed by Chisin et al., (1979) who showed presence of cochlear microphonics in nine of thirteen patients who had abnormal or no responses on ABR. He explains, since the cochlea and the brain are on the same side of the blood brain barrier the bilirubin may precipitate in the cell bodies of the cochlear neurons and thus causing damage to them, which may interfere with the normal functioning of the cochlear neurons, hence resulting in an absent response from auditory nerve. The fact that the cochlear hair cells differ in embryonic origin from neural

part of the auditory pathway (Greenfield & Majumdar, 1974), is consistent with concept of differences in susceptibility to bilirubin toxicity. Dublin (1951) also found that hair cells were normal in neonates who died following hyperbilirubinemia. Stein et al., (1996); Oyusu et al., (2001) and Berlin et al., (1997) have also reported infants having auditory dysynchrony, all with a history of hyperbilirubinemia. An animal model of bilirubin toxicity also supports the notion that auditory dysynchrony may be a sequaele of hyperbilirubinemia. Shapiro and TeSelle (1994) demonstrated that in Gunn rats, acute bilirubin concentrations can result in abnormal ABRs in the presence of normal cochlear microphonics.

Subject 1, is a very good example of compulsory serial evaluations, who demonstrated improvement in evoked potential response even long after exchange transfusion. Schwartz and Costello (1988) also reported a case of unconjugated hyperbilirubinemia who was found to have a 60 dB improvement on ABR, bilaterally. This is probably associated with delayed auditory system maturation due to transient bilirubin encephalopathy. Maturation of auditory system evolves around several mechanisms, including cell differentiation and migration, myelinisation, arborisation and synaptogenesis (Lenhardt, McArton & Bryant, 1984). These maturation processes were probably delayed in this subject due to bilirubin toxicity and prematurity. According to Lenhardt et al., (1984) hyperbilirubinemia can lead to an increase in axon degeneration and greater loss of myelin than of ciliated cells leading to a maturational delay of the auditory nerve fibers. All these factors might have led to suppressed auditory maturation in subject 1. It is evident from this subject that blood transfusion was done at birth still had some abnormalities persisting in ABR even at the age of nine months which became normal at the age of 1.5yrs. Many

researchers (Nwaesei et al., 1984; Perlman et al., 1983; Nakamura et al., 1985) have reported that the cases with neonatal jaundice, after blood transfusion, are likely to show immediate improvement in neurological status resulting in normal hearing sensitivity. However, this subject does suggest, it is not essential to observe immediate recovery of auditory functioning immediately after blood transfusion. This is in support with Bhandari et al., (1993) and Agarwal et al., (1998) who reported that treated babies showed a tendency towards recovery in ABR responses which were however not complete. Thus, in such cases, where immediate recover is not observed, follow up audiological evaluations may be useful to detect neuromaturational delay secondary to hyperbilirubinemia.

Another important observation that can be made in this group is that, four subjects in this group who demonstrated permanent damage to the auditory system had a direct bilirubin level > 1 mg/dl. Moreover, subject 1 and 2 have same total bilirubin levels but one demonstrated reversible hearing loss while other did not. The contributing factor for reversibility of hearing loss, in subject 1 could be due to low direct bilirubin levels as compared to subject 2, which were 0.7 mg/dl and 1.12 mg/dl respectively.

Therefore, a close follow up, in cases with incomplete recovery in hearing sensitivity after the treatment for jaundice, may reveal the suppressed auditory function due to neuromaturational delay. Such a recovery in auditory function even long after blood transfusion will be more probable in the presence of direct bilirubin levels < 1 mg/dl and in absence or associated with other high risk factors. However, one might notice a differential effect on the outer hair cells and auditory nerve due to bilirubin toxicity.

SUBJECT CHARACTERSTICS GROUP C: PEAK BILIRUBIN LEVEL >20 mg/dl

Subject	Age of first Evaluation / Sex	Peak Bilirubin Level	Additional Risk Factors	Auditory Behavior as observed by parents	Mode of Treatment
1	6m/M	26 mg/dl Direct- 1.6 mg/dl Indirect- 24.40 mg/dl	Premature baby	No response to auditory stimuli	Phototherapy
2	10 m/F	21.98 mg/dl Direct-1.13mg/dl Indirect-20.85 mg/dl	Low birth weight Kept in incubator	Does not respond to any auditory stimuli	Phototherapy
3	5m/M	21.4 mg/dl Direct-1.73 mg/dl Indirect-19.68 mg/dl	Premature baby Rh incompatibility	Response to auditory stimuli is inconsistent	Blood transfusion
4	4m/M	21 mg/dl Direct- 1.8 mg/dl Indirect- 19.2 mg/dl	Premature baby Kept in incubator	Response to auditory stimuli is inconsistent	Blood transfusion
5	1 m/F	39.50 mg/dl Direct -1.01 mg/dl Indirect - 38.49 mg/dl	Nil	Responses observed for moderately loud auditory stimuli	Blood transfusion

TABLE 7: Depicts the detailed case history of each subject in group C.

Case	No. of Evaln.	ABRLatencies (msec)			Intensity (dBnHL)	ABR Latencies (msec)			OAE		BOA (responses in dBnHL)			
		Right Ear				Left Ear								
		I	III	V		I	III	V	Right Ear	Left Ear	500Hz	1KHz	2KHz	Speech
1	I	N.R.			90	N.R.			Absent	Absent	N.R.	N.R.	N.R.	N.R.
	II	N.R.			90	N.R.			Absent	Absent	N.R.	N.R.	N.R.	N.R.
	III	N.R.			90	N.R.			Absent	Absent	N.R.	N.R.	N.R.	N.R.
2	I	N.R.			90	N.R.			Absent	Absent	NR	NR	N.R.	N.R.
	II	N.R.			90	N.R.			Absent	Absent	NR	NR	N.R.	N.R.
	III	N.R.			90	N.R.			Absent	Absent	NR	NR	N.R.	N.R.
3	I	N.R.			90	N.R.			Present	Present	90	95	90	70
	II	N.R.			90	N.R.			Present	Present	80	80	80	70
	III	N.R.			90	N.R.			Present	Present	85	80	80	75
4	I	N.R.			90	N.R.			Present	Present	80	85	85	80
	II	N.R.			90	N.R.			Present	Present	85	90	90	75
	III	N.R.			90	N.R.			Present	Present	85	80	80	80
5	I	N.R.			90	N.R.			Present	Present	60	75	65	60
	II	N.R.			90	N.R.			Present	Present	70	65	65	55
	III	N.R.			90	N.R.			Present	Present	60	65	75	60

TABLE 8: Depicts the ABR, OAE and BOA findings of each subject during each sitting for group C.

N.R. - No response

This group consisted of 5 subjects whose detailed information cane be seen in table 7. Subject 1, 3 and 4 were premature babies and subject 2 was reported to have low birth weight. No associated risk factors were reported for subject 5.

It can be noticed from table 8 that two out of five subjects (1 and 2) had absent ABR and OAE on the first audiological evaluation and continued to show the same on repeated evaluations. Both were high risk babies with a bilirubin level of 21.98 mg/dl for one and 26 mg/dl for the other. Other three subjects (subject 3, 4 & 5) had robust TEOAEs but showed no repeatable ABRs bilaterally to clicks at maximum intensity levels. These infants with auditory dysynchrony, when retested after three months and six months later, did not show any improvement in ABR responses. However, it can be noticed from the table 8 that these subjects exhibited behavioral responses to auditory stimuli though at higher levels suggesting better behavioral thresholds. For subject 5, these behavioral responses could be observed as low as 60 dB (for speech stimuli). This suggests the importance of BOA to trace the auditory sensitivity which was not possible based on ABR results. Estimation of behavioral threshold is important as it is a prerequisite for the selection of amplification device, if required.

Normal OAEs occurred in conjunction with neural dysynchrony as documented by the absence of a synchronous ABR. As the emissions are present in subject 3, 4 and 5, it can be assured that the functioning of OHCs is normal and middle ear is also reasonably unaffected. The absence of ABR indicated that the damage is located either in the inner hair cells, in the synapse between the IHC and the afferent dendrites or in the

fibres of the cochlear nerve. Several mechanisms to produce this disorder are: demyelinating neuropathies(Starr, Sininger, Winter, Derebery, Oba & Michalewski, 1998), synaptic receptor potential generation of the IHC, neurotransmitter liberation, absence or dysgenic lesions in the IHC or even dysfunctions of various etiologies in the brainstem or central auditory pathway (Marco, Morant, Orts, Pitarch & Garcia, 2000). Several other investigators have also reported hyperbilirubinemia to be a cause of auditory neuropathy, as already discussed in group B. Similar kind of findings were reported by Rhee et al., (1999) where four of eleven neonates with hyperbilirubinemia (peak serum bilirubin between 26.4mg/dl to 36 mg/dl) were found to have auditory neuropathy on the initial evaluation. Two of these four neonates did not demonstrate any kind of improvement in auditory function at three and six months follow up ABR. Short term episodes of hyperbilirubinemia have been shown to result in both transient and permanent ABR abnormalities, including elevated ABR thresholds (Hung, 1989) and prolonged ABR wave I – V latencies (Nakamura et al., 1985; Starr, McPherson, Patterson, Luxford et al. 1991) suggesting that both peripheral and central auditory system are vulnerable to bilirubin insult. However, all the previous studies fail to report relationship between bilirubin level and auditory neuropathy. An effort in this direction was made by Rance et al (1999). In their study auditory neuropathy occurred in ten of twenty of their cases with bilirubin concentrations greater than 20.4 mg/dl. Similar reports have been provided by Akman et al (2004) who found that seven out of nineteen infants with hyperbilirubinemia, with bilirubin level greater than 20 mg/dl, had auditory neuropathy. Anurag (2003) have reported five infants having auditory dysynchrony with a bilirubin level > 20 mg/dl. These studies support the frequency of occurrence of auditory

neuropathy seen in this group of subjects. Subject 5 in this group, having auditory dysynchrony, was free of high risk factors other than hyperbilirubinemia, such as asphyxia, prematurity and low birth weight. Hence, it is more probable that the observed aberrations in ABR, in this infant, are due to permanent toxic effect of very high level of bilirubin (39.40 mg/dl) on the brainstem nuclei.

The remaining two subjects had high levels of peak bilirubin along with other high risk factors for hearing loss which probably caused an irreversible damage to the auditory system by altering the binding properties of the bilirubin to albumin as discussed for subject 3 in group A. With regard to direct bilirubin level all the five had greater than 1mg/dl which is highly toxic to the auditory system as discussed earlier for group A and B.

Thus, it suggests that infants with serum bilirubin level > 20 mg/dl are likely to have direct bilirubin level > 1mg/dl. There is no doubt that infant with direct bilirubin level greater than 1mg/dl will suffer with neurological insult to their auditory system. However, this insult can be in the form of sensorineural hearing loss or auditory dysnchrony with varying degrees. From the above discussion one can conclude that an overall bilirubin level > 20 mg/dl which is more likely to increase the direct bilirubin level > 1 mg/dl will lead to sensorineural hearing loss or auditory dysynchrony and this is more likely when they are associated with other risk factors. The results of this study and other studies also suggests that the frequency of auditory dysynchrony is more in infants who have peak bilirubin level > 20 mg/dl

S.No.	Bilirubin level (Total & direct)	Audiological diagnosis
1	14 mg/dl Direct-0.55 mg/dl	Normal hearing
2	11.1 mg/dl Direct-0.50 mg/dl	Normal hearing
3	16.1 mg/dl Direct- 0.7 mg/dl	Reversible hearing loss
4	19.8 mg/dl Direct-1.5 mg/dl	Auditory dysynchrony
5	21 mg/dl Direct-1.8mg/dl	Auditory dysynchrony
6	21.4 mg/dl Direct- 1.73 mg/dl	Auditory dysynchrony
7	39.50 mg/dl Direct -1.01 mg/dl	Auditory dysynchrony
8	8.3 mg /dl Direct-1.0 mg/dl	Sensorineural hearing loss
9	14.3 mg/dl Direct-1.3 mg/dl	Sensorineural hearing loss
10	16 mg/dl Direct-1.12mg/dl	Sensorineural hearing loss
11	17.3 mg/dl Direct-1.62 mg/dl	Sensorineural hearing loss
12	17.2 mg/dl Direct-1.51 mg/dl	Sensorineural hearing loss
13	21.98 mg/dl Direct- 1.13 mg/dl	Sensorineural hearing loss
14	26 mg/dl Direct-1.6mg/dl	Sensorineural hearing loss

Table 9: Shows the direct and peak bilirubin levels and the audiological findings.

It can be seen in the table 9 that direct bilirubin level < 0.55 mg/dl did not show any insult to the auditory system of the newborns though they had multiple risk factors. For one subject in group B, who had a direct bilirubin level >0.55 mg/dl but < 1mg/dl, the hearing deficit returned back to normal several months after exchange transfusion indicating transient effect on the auditory system. Therefore, reversible condition can be

expected if direct bilirubin level is between 0.55 to 1mg/dl. However, it cannot be generalized as only one out of fourteen subjects had direct bilirubin within this range. Direct bilirubin levels > 1mg/dl either caused auditory dysynchrony or permanent damage to auditory system. It can also be observed from table 9 that auditory dysynchrony is seen in three patients with bilirubin level > 20mg/dl and below this level auditory dysynchrony was detected only in one subject with total bilirubin level near to 20 mg/dl i.e. 19.8 mg/dl.

Thus, it can be suggested that direct or unbound bilirubin level is a more sensitive predictor than total serum bilirubin level of ABR abnormalities seen in infants and toddlers with a history of hyperbilirubinemia with or without other associated risk factors. However, the peak bilirubin levels were also usually found to be higher in the subjects along with a functional ABR deficit that persisted even after the treatment for hyperbilirubinemia. It can also be concluded that the presence of hearing loss is more probable in infants with multiple risk factors as compared to those with a single risk factor. The bilirubin toxicity can affect the auditory nerve fibers differentially or can cause damage to both auditory nerve fibers and the outer hair cells, which probably depends on other factors.

Another point to be mentioned is that if a suspicion of hearing loss is established with ABR, in the diagnostic phase it should be mandatory to perform OAE test in order to establish whether the mechanical function of outer hair cells is maintained or not, because, if auditory dysynchrony is detected the clinical characteristics and treatment of

this disorder will differ from other causes of hearing loss in infants. As compared to cases with severe hearing loss, cases with auditory dysynchrony exhibit behavioral responses, though at higher intensity levels. However, as OAE and ABR do not give an estimate of hearing sensitivity in cases with auditory dysynchrony, BOA responses should be considered to estimate the behavioral responses. Hence, behavioral observation audiometry responses are very essential in such cases. Also if case of auditory dysynchrony is detected a close follow up should be performed using a test battery approach in order to assess more precisely the auditory threshold which can improve over time owing to some kind of maturational delay and thus help in selection of the best therapeutic approach. Therefore, a test battery approach with simultaneous use of ABR, OAE and BOA is essential in the infants with high risk factors

SUMMARY AND CONCLUSIONS

Besides other sequaele, hyperbilirubinemia is particularly toxic for the auditory pathway and may result in auditory dysynchrony with varying degrees of hearing loss or permanent sensorineural hearing loss. ABR has been most commonly employed to investigate the auditory status related to hyperbilirubinemia which, in cases of hyperbilirubinemia, is characterized by increased threshold, increased latency, and reversibility after exchange transfusion and correlation to peak serum bilirubin level. Presence of OAEs in this population suggests that the main site of lesion is neural rather than cochlear. Though it has been established that infants with neonatal hyperbilirubinemia may have sensorineural hearing loss or auditory neuropathy due to bilirubin toxicity, literature regarding continuous electrophysiological and behavioral monitoring on these infants is scarce. Some kind of maturational delay might be speculated in this group of high risk babies and hence untimely management of these infants can lead to amplification induced hearing loss, thus compromising the child's future developmental course.

Hence , the present study was taken up to investigate the auditory status of infants and toddlers with hyperbilirubinemia and physiological jaundice using ABR, OAE and BOA to document the pathophysiological changes that occur in association with neonatal hyperbilirubinemia. Total fourteen infants in the age range of one month to nine months with a history of neonatal physiological jaundice or neonatal hyperbilirubinemia were tested longitudinally after approximately three months till three audiological evaluations

were completed. These subjects were divided into three different groups based on there peak serum bilirubin level: group A (peak bilirubin level <15 mg/dl), group B (peak bilirubin level 15 – 20 mg/dl) and group C (peak bilirubin level > 20 mg/dl). Group A consisted of four infants and five infants in both group B and group C. Single channel ABR recording wa done for 1500 clicks presented at a rate of 11.1 / sec with a filter setting of 30 Hz to 3 KHz and time window of 15 msec. The presentation level of the stimulus was decided based on the behavioral observation audiometry results. The potential was recorded using Nicolet Bravo version 3 and ILO DP echoport was used to record TEOAEs using a nonlinear paradigm. The direct and indirect level of bilirubin present during the hyperbilirubinemia and other associated high risk factors present during neonatal period were also recorded.

The results on ABR, OAE and BOA were first compared within each group and then between groups and therefore trying to draw a relationship between hearing loss and the level of bilirubin (peak level and direct level). The speculation of results of audiological testing of these groups revealed that bilirubin toxicity has a differential effect on cochlear hair cells and auditory nerve in some, if not all, infants who had neonatal hyperbilirubinemia. Four of fourteen infants had auditory dysnchrony out of which three were in group C and one in group B. There follow up audiological evaluation did not show any kind of improvement in the auditory skills. Three out of five in group B and two out of five in group C were found to have irreversible bilateral severe hearing loss. Only one infant in group B showed an improvement in hearing sensitivity which can be attributed to a lower level of direct bilirubin level (0.70 mg/dl). Other infants who had

severe hearing loss had a direct bilirubin level > 1mg/dl. Two infants in group A were found to have normal hearing and had direct bilirubin level within 0.55 mg/dl but the other two, who were found to have bilateral severe hearing loss, with a direct bilirubin level > 1mg /dl along with multiple high risk factors. However, risk factors like low birth weight not necessarily cause an insult to the auditory system rather it affecting the albumin and bilirubin binding capacity resulting in increased direct bilirubin levels which is highly toxic to the auditory system. Thus, a strong relationship is found between direct bilirubin levels and hearing status rather than peak serum bilirubin levels which can be seen in table 10.

Direct Bilirubin Level(mg/dl)	**Auditory Status**
< 0.6	Expected to have normal hearing.
> 0.6 to1	Reversible condition can be expected if suspected to have hearing loss in initial recording.
> 1	Can lead to auditory dysynchrony or permanent severe sensorineural hearing loss.

Table 10: Depicts the relationship between direct bilirubin level and its effect on the auditory system

CONCLUSION

High levels of direct bilirubin levels are more toxic to the auditory system even in the presence of lower peak serum bilirubin levels. High levels of peak serum bilirubin

levels in the absence of any associated risk factors might render the auditory system susceptible to damage to the same extent as lower levels of bilirubin in the presence of multiple risk factors. The toxicity due to bilirubin has a differential effect on hair cells and auditory nerve fibers which might also result in suppressed maturation of auditory system. A close follow up, in cases with incomplete recovery in hearing sensitivity after the treatment for jaundice is required, which might reveal the suppressed auditory function due to delayed neuromaturation. A test battery approach with simultaneous use of ABR, OAE and BOA is essential in the infants with high risk factors

IMPLICATIONS

- Direct bilirubin level should be included in high risk register rather than peak bilirubin level.

- A complete assessment using a test battery approach is mandatory for all infanst and toddlers with suspicion of hearing loss.

- Follow up is very important of cases suspected to have hearing loss due to hyperbilirubinemia as one can expect reversible conditions.

- For rehabilitation a close observation of auditory behaviour is very important before amplification is provided as it might lead to permanent loss due to overamplification in cases where reversible condition is expected.

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