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Original Article

Physiology Section

Brainstem Auditory Evoked Potential in Common Causes of Neonatal Haemolytic Hyperbilirubinaemia- A Cross-sectional Study

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ABSTRACT

Introduction: Neonatal haemolytic hyperbilirubinaemia often leads to multiple neurological consequences. There are various haemolytic causes among which ABO incompatibility, Rh incompatibility, G6PD deficiency are much common in Indian population. High concentration of serum Unconjugated Bilirubin (UCB) is lethal for the auditory pathway as that delays sound transmission and causes speech problem in later life. Though, for infant population no subjective test of hearing is dependable, auditory assessment can be done by Brainstem Evoked Response Audiometry (BERA) that can measure Brainstem Auditory Evoked Potential (BAEP) among children reliably.

Aim: To assess the effect of neonatal haemolytic hyperbilirubinaemia on auditory pathway by BERA and compare Auditory Brainstem Response (ABR) in different haemolytic groups (Rh incompatibility, ABO incompatibility, G6PD deficiency, other haemolytic causes like hereditary spherocytosis, elliptocytosis etc.

Materials and Methods: This cross-sectional analytical study was commenced in Applied Neurophysiology laboratory of Department of Physiology, RG Kar Medical College, Kolkata, West Bengal, India from July 2019 to December 2024. Altogether 96 infants, suffering from neonatal hyperbilirubinaemia due to different haemolytic causes were taken from Department of Paediatric Medicine. The total population was categorised into four groups as per causes i.e., ABO incompatibility, Rh incompatibility, G6PD deficiency and other haemolytic causes (spherocytosis, elliptocytosis etc.,). The BAEP wave latencies of case population was compared with normative data, taken from previous study. Intergroup and intragroup comparison was done by student's t-test, Hedges'g value, Analysis of Variance (ANOVA) test and post-hoc test.

Results: In the present study, out of total 96 subjects, there were 58 male and 38 female infants with mean age of 1.98±0.87 months. All the subjects had bilateral statistically significant delayed wave V and I-V interwave latency. Though as per effect size, highly significant difference was present in right-sided waves. ABO incompatibility, Rh incompatibility, and other haemolytic causes showed similar BAEP findings though the parameters delay was more significant in Rh incompatibility in comparison with other three groups. In G6PD deficiency, only right-sided wave I was delayed. A total of 26 infants showed unilateral or bilateral absent waves, while maximally it was present in G6PD deficient group.

Conclusion: Infants with neonatal haemolytic hyperbilirubinaemia, alongside the three aforementioned groups, showed positive association between high serum bilirubin and prolonged central auditory pathway conduction; though in G6PD deficient infants, only the right-sided peripheral pathway was impacted, while infants with Rh incompatibility showed a more significant involvement of the right-sided central auditory pathway than the other three groups.

Keywords: Auditory effect, Bilirubin toxicity, Erythrolysis, Newborn jaundice, Unconjugated bilirubin

INTRODUCTION

Hyperbilirubinaemia is a common issue in the neonatal life. Severe indirect hyperbilirubinaemia may be neurotoxic if left untreated. About 60% of term newborns as well as 80% of premature babies, experience jaundice during the first week of life [1]. The deposition of unconjugated, non-polar, lipid-soluble bilirubin pigment from haemoglobin in the skin typically causes the colour. Under particular circumstances and at specific quantities, the unconjugated form is lethal to an infant's nervous system [2]. Haemolysis caused by both immune and non-immune mechanisms increases bilirubin and is considered, the commonest cause of unconjugated pathological hyperbilirubinaemia [3].

Generally, maturation of bilirubin conjugation and elimination happens following the first day of neonatal period [4]. The common causes of Immune-mediated haemolysis are ABO, Rh, or other RBC antigen incompatibility. The non-immune haemolysis occurs mostly in deficiencies of Red Blood Cells (RBC) enzymes like G6PD deficiency or RBC membrane defect like hereditary spherocytosis, elliptocytosis haemoglobinopathies etc., [5]. Elevated serum levels of UCB are considered toxic for the auditory pathway and the central nervous system, and are included among the risk factors for neonatal deafness and encephalopathy. A high concentration of biliary pigments is treated with phototherapy and/or exchange transfusion; the latter is reserved for severe cases and those resistant to repeated phototherapy [6,7].

Multiple works done in past years showed auditory involvement (17-87%) among children with hyperbilirubinaemia [8-11]. The detrimental effect of hearing pathway involvement is prolonged as speech development is very much dependent upon auditory capability [12]. So, hearing screening is therefore essential to detect early pathology. BERA is an important objective test that can assess bilirubin toxicity in auditory pathway as well as its functional

integrity and maturation as subjective methods are not useful to evaluate this ototoxicity in such early period of life [13]. In this test, the electrical activity of auditory pathway is measured by providing proper acoustic stimulus [14]. Recording of BERA consists of five waves, among which waves I, III, V are prominent and wave V is the most consistent one. Wave I latency and I-V interwave latency are also two important parameters as the delay of first one is related to pathology present in peripheral auditory pathway and delay of the later, signifies, central auditory pathway involvement. When a population of neurons fire together in synchrony, proper wave is generated. If, neuronal pathway is affected by deposition of any toxic material like free bilirubin, synchronous firing will be disrupted and there will be prolonged latency or absent waves [15].

Though there are many studies [8-11], showing relation between high bilirubin and BAEP changes, there are paucity of researches, stating BAEP abnormalities in individual causes of high bilirubin. Few researchers found more neuronal abnormality in Rh disease [16,17], while a different observation showed ABO incompatibility is maximally related with BAEP changes [18], another study reported association between G6PD deficiency and altered neuronal outcome [19]. However; there are contradictory views among previous researchers [16-19], in this scenario, the present study was done to assess its consequence in more precise way.

The current cross-sectional study was done to assess the effect of neonatal haemolytic hyperbilirubinaemia in BERA response and to compare BAEP changes among different haemolytic causes (Rh incompatibility, ABO incompatibility, G6PD deficiency, other haemolytic causes like hereditary spherocytosis, elliptocytosis etc.

MATERIALS AND METHODS

This cross-sectional analytical study was commenced in Applied Neurophysiology laboratory of Department of Physiology at RG Kar Medical College, Kolkata, West Bengal, India from July 2019 to December 2024, after getting approval from Institutional Ethics Committee (RKC/295 dated 15/1/19).

Inclusion criteria: The infants diagnosed with neonatal haemolytic hyperbilirubinaemia who were admitted to the Neonatal Intensive Care Unit (NICU for inborn), Paediatric Intensive Care Unit (PICU for outborn) and referred cases from sick baby clinic to the Department of Paediatric Medicine at RG Kar Medical College and Hospital, Kolkata, were included. All included cases had their guardians who provided written informed consent.

Exclusion criteria: Infants born preterm, with H/O low Birth Weight (BW), in acute stage of hyperbilirubinaemia undergoing intervention, very sick children, infants of unwilling guardian or parents, maternal infection in first trimester of pregnancy, birth trauma, asphyxia, meningitis or infants with any brain abnormality or external ear pathology or suffering from Upper Respiratory Tract Infection (URTI) at the time of test were excluded.

Sample size calculation: About 10.4% of infants suffering from haemolytic hyperbilirubinaemia had BAEP abnormality in a previous study [18].

$N = Z^2 . p(1-p)/d^2$

$= 1.96x1.96x\ 0.1x0.9/.06x.06$

$= 96.04$

Therefore, 96 cases were selected where Z=1.96, p=0.1, d (margin of error) =0.06

Data Collection

A total number of 96 infants with history of neonatal haemolytic hyperbilirubinaemia were tested and BAEP findings were recorded. The value was compared with 30 age-matched normal infants. The normative data was taken from previous study, which was done in year 2016-17, with same set-up [20]. Test was done in sleeping infants with no use of sleep inducing medications.

The cases were further categorised into four groups according to reasons of haemolysis and each group was compared with control group as well as comparison among groups were done. The groups were Group 1 (n=24) infants with H/O ABO incompatibility, Group 2 (n=26) infants with H/O Rh incompatibility, Group 3 (n=22) infants with H/O G6PD deficiency, Group 4 (n=24) infants with H/O other haemolytic pathology (spherocytosis, elliptocytosis etc.,). These infants were recommended BERA test in Applied Neurophysiology Lab at Department of Physiology in next three months of their date of birth.

The BERA, that can assess BAEP parameters were measured through the instrument Neuro-MEP4. NET (version 3) Ivanovo, Russia. At first, clinical history was elicited and case record forms were filled after parents' interview. External ear examination was done before the actual test. Parents were informed about the study and instructed to apply shampoo in infants' hair one day prior to the test. On the day of examination, the study details were explained fully to guardians and written informed consent was taken. The guardians were also requested for early awakening of infants as natural sleep during the whole recording time is very important to eliminate biologically induced noise. Though awake, but, restful and quiet child were also accepted.

For recording of BERA, surface electrodes were used. Electrolyte paste was applied to fix these silver cup electrodes. The electrode impedance was kept below 5 kilo-ohm. One electrode was positioned at the vertex (Cz) and two electrodes were set at two earlobes following International 10-20 system. The ipsilateral earlobe where stimulus was applied marked as Ai and contralateral earlobe was marked as Ac. Fz, the ground electrode was set at the forehead. Monophasic click stimulus of 0.01 ms duration, 0.5 microvolt sensitivity and 11.1 Hz rarefaction polarity was applied in sleeping infants with adjusted filter 30-1000Hz. Coronavirus Disease 2019 (COVID-19) safety protocol was ensured properly. Ipsilateral ear was exposed to 70 dB Sound Pressure Level (SPL) clicks, while 30 dB SPL sound were used for contralateral side in the form of masking noise with headphone. In each ear, two recordings containing average 2000 evoked responses were taken to ensure reproducibility [15].

In BAEP recording, waves I, III, V are significant waves among infants. In present study, absolute latencies of waves I, III, V and I-V interwave latency were compared between case and control population. The delayed latencies or the absent waves both were regarded as atypical with probable presence of pathology in auditory pathway.

STATISTICAL ANALYSIS

The parameters were compared between 96 infants with neonatal haemolytic hyperbilirubinaemia and 30 healthy infants included as control population. Same comparison study was done between each subgroup and control group. Student's t-test was applied in both the cases and Fisher-Exact test was applied for qualitative parameter (sex) comparison. Graph Pad Quick Calc software, California, USA was used to analyse the result. Effect size, that can quantify the magnitude of difference between groups, was calculated with Hedges'g value. Intragroup comparison was done by using

One-way ANOVA test, which is followed by Tukey HSD post-hoc test to assess statistically significant difference between two groups among all four groups. Results were statistically analysed with http://www.statpages.info software, USA. The p-value <0.05 was taken as statistically significant.

RESULTS

This study included 96 infants with haemolytic hyperbilirubinaemia of different causes. Normative data of the control population of 30 healthy infants of similar age and sex were also taken from a former study. The mean age of infants was 1.98(.87) months, mean BW was 2.67(0.23) kg, mean highest bilirubin level was 21.36(4.32) mg% or mg/dL. In comparison of the demographic data, between total cases and control, Student's t-test (for quantitative parameters), and Fisher-Exact test (for qualitative parameter), both showed no statistically significant difference between groups. Hence, they were matched as per age, sex and body weight [Table/Fig-1].

Groups	Mean age (Months)	Mean BW (Kg)	Gender distribution Male/ Female	Mean±SD bilirubin level
ABO incompatibility	2±0.83	2.72±0.31	11/13	20.58± 4.83
Rh incompatibility	1.92±0.98	2.61±0.14	16/10	23.03± 4.46
G6PD deficiency	2.09±0.97	2.75±0.27	15/7	21.23± 4.38
Other cause related neonatal hyperbilirubinaemia	1.92±0.88	2.6±0.13	16/8	20.44± 3.16
Total cases of neonatal haemolytic Hyperbilirubinaemia	1.98±0.87	2.67±0.23	58/38	21.36± 4.32
Control group	2±0.91	2.66±0.17	21/9	
p-value	0.91	0.83	0.39	

[Table/Fig-1]: Distribution of age, Birth Weight (BW), sex and bilirubin level among groups.

All the infants were subjected to BERA, and wave I, III, V latencies and I-V interwave latency were recorded and compared between cases and controls, between subgroups and controls and also among subgroups. In the current study, all the four groups showed some amount of absence of waves that is depicted in [Table/Fig-2].

Groups	Total subjects	Number of cases with absent waves	Number of only Right-sided absent waves	Number of only left-sided absent waves	Number of bilateral absent waves
ABO incompatibility	24	4 (16.67%)	0	2	2
Rh incompatibility	26	8 (30.77%)	2	0	6
G6PD deficiency	22	10 (45.45%)	5	2	3
Other cause related neonatal hyperbilirubinaemia	24	4 (16.67%)	2	0	2

[Table/Fig-2]: Distribution of absent waves among groups.

In both ears, the latencies of wave V and the interwave latencies of I-V were significantly delayed in cases. This was suggestive of neurotoxic damage of central auditory pathway and increased Central Transmission Time (CTT). In left-side all the waves were absent in 17 cases and in right side all the waves were absent in 22 cases. A moderate effect size was present for wave V, I-V interwave latency between cases and controls in the left ear, and a large effect size was present for wave V, I-V interwave latency between cases and controls in the right ear. Therefore, with respect to central auditory pathway involvement, a significant difference was present in the left ear, and a highly significant difference was present in the right ear among cases than the control group [Table/Fig-3a,3b].

BAEP parameters latency (mS)	Case Mean (SD) (n=79)	Control Mean (SD) (n=30)	p-value	Hedges' g of significant parameters
Wave I	1.94±0.25	1.9±0.14	0.41	
Wave III	4.54±0.5	4.57±0.28	0.76	
Wave V	6.87±0.53	6.63±0.21	0.02*	0.52
I-V interwave latency	4.93±0.49	4.74±0.17	0.04*	0.44

[Table/Fig-3a]: Comparison of BAEP parameters in left ear between cases (neonatal haemolytic hyperbilirubinaemia, total n=96) and controls. *Statistically significant (Student's t-test applied)

Wave V and I-V interwave latencies were statistically significantly prolonged in both sides among ABO incompatibility, causing hyperbilirubinaemia in comparison with the control group infants [Table/Fig-4], suggestive of bilirubin mediated damage affecting central auditory pathway.

In haemolytic hyperbilirubinaemia due to Rh incompatibility, wave V and I-V interwave latencies were statistically significantly prolonged in both the ears with delayed wave III in right ear only [Table/Fig-5] and that suggests neurotoxic damage of central auditory pathway in both the sides but in right-side it's affecting both the parts of the pathway i.e., anywhere up-to superior olivary complex and beyond that, up-to inferior colliculus.

In haemolytic hyperbilirubinaemia due to G6PD deficiency, when compared with control group, no waves were statistically significantly prolonged in the left ear and in the right ear, wave I was statistically significantly prolonged, as was wave V but I-V interwave latency were Within Normal Limit (WNL) [Table/Fig-6]. This was suggestive of bilirubin mediated damage affecting right-sided peripheral auditory pathway.

Wave V and I-V interwave latencies were statistically significantly prolonged in both sides in haemolytic hyperbilirubinaemia due to other causes; in comparison with the control group infants [Table/Fig-7], suggestive of bilirubin mediated damage affecting central auditory pathway.

In intragroup comparison, p-value of right-sided wave III, wave V and I-V interwave latency was indicative of significant statistical difference among four groups [Table/Fig-8]. Tukey HSD post-hoc was done to indicate which groups were significantly different from which others with respect to aforementioned three BAEP parameters. Wave III latency of the right ear was statistically significantly different between Rh incompatibility and ABO incompatibility and Rh incompatibility and other causes. The statistically significant difference is also present between ABO incompatibility and other causes [Table/Fig-9]. Wave

BAEP parameters latency (mS)	Case Mean (SD) (n=74)	Control Mean (SD) (n=30)	p-value	Hedges' g of significant parameters
Wave I	1.98±0.29	1.9±0.14	0.15	
Wave III	4.62±0.46	4.54±0.36	0.35	
Wave V	6.87±0.32	6.58±0.17	0.0001*	1.02
I-V interwave latency	4.9±0.34	4.68±0.16	0.001*	0.73

[Table/Fig-3b]: Comparison of BAEP parameters in right ear between cases (neonatal haemolytic hyperbilirubinaemia infants, total n=96) and controls.
*Statistically significant

Ear	BAEP parameters latency (mS)	Case Mean (SD)	Control Mean (SD) (n=30)	p-value
Left ear (n=20)	Wave I	1.95±0.19	1.9±0.14	1
	Wave III	4.62±0.5	4.57±0.28	0.58
	Wave V	6.84±0.21	6.63±0.21	0.001*
	I-V interwave latency	4.9±0.29	4.74±0.17	0.02*
Right ear (n=22)	Wave I	1.95±0.24	1.9±0.14	0.35
	Wave III	4.48±0.39	4.54±0.36	0.57
	Wave V	6.79±0.18	6.58±0.17	0.0001*
	I-V interwave latency	4.84±0.24	4.68±0.16	0.006*

[Table/Fig-4]: Comparison of BAEP parameters in both ears between cases (neonatal hyperbilirubinemic infants due to ABO incompatibility, total n=24) and controls.
*Statistically significant

Ear	BAEP parameters latency (mS)	Case Mean (SD)	Control Mean (SD) (n=30)	p-value
Left ear (n=20)	Wave I	1.93±0.25	1.9±0.14	0.59
	Wave III	4.59±0.59	4.57±0.28	0.87
	Wave V	7.02±0.59	6.63±0.21	0.002*
	I-V interwave latency	5.05±0.53	4.74±0.17	0.004*
Right ear (n=18)	Wave I	1.86±0.18	1.9±0.14	0.39
	Wave III	4.93±0.36	4.54±0.36	0.0007*
	Wave V	7.1±0.2	6.58±0.17	0.0001*
	I-V interwave latency	5.24±0.3	4.68±0.16	0.0001*

[Table/Fig-5]: Comparison of BAEP parameters in both the ears between cases (neonatal hyperbilirubinemic infants due to Rh incompatibility, total n=26) and controls.
*Statistically significant

Ear	BAEP parameters latency (mS)	Case Mean (SD)	Control Mean (SD) (n=30)	p-value
Left ear (n=17)	Wave I	1.94±0.23	1.9±0.14	0.46
	Wave III	4.49±0.51	4.57±0.28	0.48
	Wave V	6.68±0.57	6.63±0.21	0.67
	I-V interwave latency	4.74±0.54	4.74±0.17	1
Right ear (n=14)	Wave I	2.12±0.33	1.9±0.14	0.004*
	Wave III	4.68±0.33	4.54±0.36	0.22
	Wave V	6.76±0.23	6.58±0.17	0.006*
	I-V interwave latency	4.64±0.18	4.68±0.16	0.46

[Table/Fig-6]: Comparison of BAEP parameters in both the ears between cases (neonatal hyperbilirubinemic infants due to G6PD deficiency, total n=22) and controls.
*Statistically significant

Ear	BAEP parameters latency (mS)	Case Mean (SD)	Control Mean (SD) (n=30)	p-value
Left ear (n=22)	Wave I	1.95±0.32	1.9±0.14	0.45
	Wave III	4.47±0.42	4.57±0.28	0.31
	Wave V	6.92±0.61	6.63±0.21	0.02*
	I-V interwave latency	4.97±0.56	4.74±0.17	0.04*
Right ear (n=20)	Wave I	2.03±0.35	1.9±0.14	0.07
	Wave III	4.45±0.37	4.54±0.36	0.39
	Wave V	6.84±0.46	6.58±0.17	0.007*
	I-V interwave latency	4.83±0.35	4.68±0.16	0.045*

[Table/Fig-7]: Comparison of BAEP parameters in both the ears between cases (Infants with neonatal hyperbilirubinaemia due to other causes, total n=24) and controls.
*Statistically significant

V latency and I-V Interwave latency was statistically significantly different between Rh incompatibility and ABO incompatibility; Rh incompatibility and other causes as well as Rh incompatibility and G6PD deficiency [Table/Fig-9]. This was suggestive of more central auditory pathway involvement in the case of Rh incompatibility than the other three groups.

DISCUSSION

In current study, click BERA was performed to assess BAEP parameters between 96 infants with neonatal haemolytic hyperbilirubinaemia undergoing phototherapy or exchange transfusion and 30 healthy infants with similar age, as control group. The study was commenced to see the association between high serum bilirubin and auditory pathway involvement. The study showed prolonged wave V and I-V interwave latency in both the ears in case population which is suggestive of higher level of bilirubin corresponds with higher involvement of central auditory pathway, mainly near both sided inferior colliculus (midbrain). The cases are further divided into four groups based on primary causes of haemolysis of neonatal period. The first group i.e., ABO incompatibility and the fourth group, i.e., hyperbilirubinaemia, due to other haemolytic causes, infants showed same central auditory pathway involvement involving inferior colliculus areas of both sides. The second group i.e., Rh incompatibility, cases having central auditory pathway pathology too, but here toxicity involved areas around inferior colliculus as well as superior olivary complex (pons) of both sides. The third group, i.e., G6PD deficiency, infants showed no pathology in left-side but right-sided BAEP finding was suggestive of noticeable upward trend between higher bilirubin level and peripheral auditory pathway involvement. Here, delayed wave V was due to delayed sound transmission through peripheral part (as wave I latency was delayed too) and it's not due to pathology of central pathway as I-V interwave latency is not prolonged [15]. But when intragroup comparison was done, Rh incompatible group showed maximum bilirubin mediated central pathway conduction delay than other groups. Probably the reason behind this is in Rh incompatibility, haemolysis and bilirubin production was more (average level 23.03 mg/dL) than the other groups (20.58mg/dL in ABO incompatibility, 21.23mg/dL in G6PD deficiency, 20.44mg/dL in other haemolytic causes) [21]. Besides antenatal care to prevent such condition was also inadequate [22,23]. Among 96 of total case population 26 infants (27%) showed absent waves in one or both the ears.

The risk of neuronal injury by bilirubin is primarily determined by the concentration of unbound or "free" unconjugated bilirubin

BAEP parameters	Groups Mean (SD)					
	ABO Incomp.	Rh Incomp.	G6PD Def.	Other haemolytic causes	F value	p-value
Lt I	1.95±0.12	1.93±0.25	1.94±0.23	1.95±0.32	0.03	0.99
Rt I	1.95±0.24	1.86±0.18	2.12±0.33	2.03±0.35	2.56	0.06
Lt III	4.62±0.5	4.59±0.59	4.49±0.51	4.47±0.42	0.43	0.73
Rt III	4.48±0.39	4.93±0.36	4.68±0.33	4.45±0.37	6.91	0.0004*
Lt V	6.84±0.21	7.02±0.59	6.68±0.57	6.92±0.61	1.39	0.25
Rt V	6.79±0.18	7.1±0.2	6.76±0.23	6.84±0.46	4.92	0.004*
Lt I-V	4.9±0.29	5.05±0.53	4.74±0.54	4.97±0.56	1.31	0.28
Rt I-V	4.84±0.24	5.24±0.3	4.64±0.18	4.83±0.35	13.62	<0.001*

[Table/Fig-8]: ANOVA test for intragroup comparison of all the BAEP parameters.

BAEP Parameters	Group	Vs	Group	p-value
Right III	ABO incomp.	Vs	Rh incomp.	0.001*
	ABO incomp.	Vs	G6PD Def.	0.39
	ABO incomp.	Vs	Other	0.03*
	Rh incomp.	Vs	G6PD Def.	0.23
	Rh incomp.	Vs	Other	0.0008*
	G6PD Def.	Vs	Other	0.28
Right V	ABO incomp.	Vs	Rh incomp.	0.008*
	ABO incomp.	Vs	G6PD Def.	0.99
	ABO incomp.	Vs	Other	0.95
	Rh incomp.	Vs	G6PD Def.	0.01*
	Rh incomp.	Vs	Other	0.04*
	G6PD Def.	Vs	Other	0.86
Right I-V	ABO incomp.	Vs	Rh incomp.	0.0002*
	ABO incomp.	Vs	G6PD Def.	0.17
	ABO incomp.	Vs	Other	0.99
	Rh incomp.	Vs	G6PD Def.	<0.001*
	Rh incomp.	Vs	Other	0.0001*
	G6PD Def.	Vs	Other	0.23

[Table/Fig-9]: Post-hoc Test of intragroup comparison of Right III, V wave latencies and Right I-V interwave latency.

(Bf) and hydrogen ion (pH) in blood. UCB enters brain tissue as Bf when the blood's bilirubin-binding capacity is exceeded [24]. In the auditory system, bilirubin does not appear to affect either inner or outer hair cells, although it may affect the cell bodies of the auditory nerve in the spiral ganglia. The most sensitive area in the auditory system seems to be in the brainstem auditory nuclei. Bilirubin encephalopathy affects superior olive, lateral leminiscus and inferior colliculus [25]. The auditory pathways in the thalamus and cortex do not seem to be affected. These auditory brainstem nuclei cannot be imaged with currently available techniques, but can be assessed neurophysiologically. The mechanical structure of the inner ear is assessed clinically with Otoacoustic Emissions (OAEs), and the outer hair cells of the inner ear are tested with Cochlear Microphonic responses (CMs). Both OAEs and CMs are normal in neonates with bilirubin-induced injury. The ABR, a.k.a. BAEP or response (BAER), is absent or abnormal, reflecting damage to the auditory nerve (wave I) and/or, more likely, auditory brainstem nuclei (waves III and V) [24]. Many workers had presented the relation between hyperbilirubinaemia and BAEP abnormalities.

Lenhardt ML et al., performed BAEP testing two times in 10 neonates with hyperbilirubinaemia found that absolute wave III and wave V latencies were higher in the study group, compared with the control group [26]. Nakamura H et al., carried out BAEP testing pre and post-treatment in 56 hyperbilirubinemic neonates and 24 control [27]. Absolute Wave I latency, prolonged in pre-treatment test, getting corrected post-exchange transfusion while I-V interwave latency delay persisted even after treatment.

Agarwal V et al., Sharma P et al., Sabatino G et al., Rhee CK et al., Nwaesei CG et al., all performed BAEP either pre or post treatment or carried on a follow-up study [21,28-31]. In these studies, there was partial improvement of BAEP parameters in some children while among the rest abnormality persisted. There are multiple cross-sectional studies like current study. Salehi N et al., showed the BAEP findings among 42 term hyperbilirubinaemic infants and got significant increase in the absolute latencies of waves III and V, and I-III and I-V inter-peak latencies of the sample group compared to the control group in both ears [32]. De Silva DPC and Martins RHG showed that BAEP findings of 25 hyperbilirubinaemic infants were, significant delay of wave V in right ear & IV interwave latency in left ear [10].

Mukhopadhyay K et al., showed, prolonged BAEP waves in 76% cases among 25 term newborn with Total Serum Bilirubin (TSB) >20mg/dL, treated with exchange transfusion [33]. BERA test was done at three months of age similar to the present study. In these researches, BAEP findings were like delayed wave V latency, or I-V interwave latency or increased threshold or absent waves. Therefore, it's observed that in most of the studies, most affected part is central auditory pathway and not the peripheral one just like this study. Though in current study peripheral part was affected also but only in G6PD deficient group. There was a contradictory view too. Chen WX et al., found no significant association between the maximum total serum bilirubin and parameters of BAEPs [13]. There were very few studies showing BAEP changes among individual causes of hyperbilirubinaemia.

Wong VCN et al., performed BAEP test among 128 neonates with increased bilirubin to see effects of haemolytic hyperbilirubinaemia on the auditory brainstem pathway [18]. These children were divided into two groups: a haemolytic group and a non-haemolytic group. At the initial assessment BAEP abnormalities occur in 10.4% in the haemolytic cases due to all related to ABO incompatibility and 9.1% in the non-haemolytic group. Wennberg RP et al., reported a case of a neonate with hyperbilirubinaemia due to Rh incompatibility, TSB more than 15 mg/dL, in whom follow up BAEP testing was done within 27 hours of life, after exchange transfusion, and at three months of age [17]. Though there was improvement of BAEP threshold in subsequent recordings, prolongation of left I-V interwave latency persisted since first assessment, suggestive of retrocochlear auditory involvement.

Other studies also showed that individual causes can affect neurodevelopmental status of infants. Bushra M et al., showed

strong correlation between Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency and neonatal hyperbilirubinaemia with a rare but potential threat of devastating acute bilirubin encephalopathy [19]. Bhutani VK et al., showed that, Rh disease, is a cause of neonatal mortality and long-term neurodevelopmental impairment [16].

In each group, some infants' recordings showed absent waves. It happens either due to desynchronisation of electrical signal or irregular activity of neuron in peripheral and central hearing pathway followed by problem in summation and therefore no identifiable waveform was produced. Same pathological inconsistency can produce delay or absent waves. Pathology of peripheral part only can generate loss of entire waveforms. Though precise location of pathology cannot be determined absent wave means presence of auditory dysfunction somewhere in the pathway. However, it does not mean non transduction of auditory signal, but disturbance in the process [15].

Limitation(s)

The cross-sectional design of the current study was one main hindrance. The study quality would improve if the test was done before and after treatment and followed up in different time of childhood to know improvement or consistency of delayed wave and to assess pattern of speech development. Though that was the initial idea, due to high dropout in follow up studies, that plan was cancelled. The larger sample size and larger and same number of control population were also made the study better. The other details like maternal comorbidity, previous birth history, family history, drug history, environmental factor, other antenatal history were also insufficient.

CONCLUSION(S)

Neonatal hyperbilirubinemic infants showed strong positive correlation between high serum bilirubin and bilateral central auditory pathway involvement. As per causes of neonatal haemolytic hyperbilirubinaemia, ABO incompatibility, Rh incompatibility, and other causes responsible; were associated with same bilateral central auditory pathway transmission delay while only G6PD deficiency was related with prolonged right peripheral auditory pathway conduction. Though maximum number of absent wave cases was found in this group, suggestive of derangement of auditory transmission but location of pathology is inconclusive. Among various haemolytic causes, Rh incompatibility demonstrated a notably greater delay in right-sided CTT probably due to highest bilirubin level. Consequently, the findings of this study provided essential insights into the BAEP abnormalities observed in haemolytic hyperbilirubinemic infants and indicated which of the common haemolytic causes has a more significant impact than others.

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1st Author- Conceptualisation, Investigation, Supervision, Data collection, 2nd Author (corresponding)- Resources, Data curation, Analysis, Writing.

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