Article

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Clinical Analysis of Abnormal Brainstem Auditory Evoked Potential in Neonates with Hyperbilirubinemia

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Background: Severe neonatal hyperbilirubinemia can cause hearing impairment. Bilirubin can be deposited in nerve cells, and the brainstem and the 8th nerve are especially sensitive to bilirubin toxicity. Abnormal changes in brainstem auditory evoked potential (BAEP) can be observed, and the BAEP test measures a nerve potential induced by short, high-frequency sound stimulation; thus, it is able to detect damage to the auditory conduction pathway in children. We aimed to identify relationships between clinical features and BAEP abnormalities in children with hyperbilirubinemia and to assess the predictive power of these risk factors for bilirubin-induced neurological damage.

Methods: Children with hyperbilirubinemia were evaluated with BAEP and retrospectively enrolled in the study between January 2012 and December 2018. Multivariate logistic regression was performed to identify independent predictors of BAEP abnormalities.

Results: Of the 561 children with hyperbilirubinemia enrolled, the BAEP anomaly group accounted for 198 (35.3%) cases. Except for body weight, there were no significant differences in the general data between the two groups with hyperbilirubinemia ($p > 0.05$). Univariate analysis showed that prematurity, abnormal umbilical cord, and gestational diabetes during pregnancy were significantly correlated with abnormal BAEP. Multivariate logistic regression analysis identified prematurity ($p = 0.001$), gestational diabetes ($p = 0.03$), Premature rupture of membranes ($p = 0.013$), total serum bilirubin (TSB), bilirubin/albumin (B/A) as independent risk factors for BAEP abnormalities. The prediction accuracy of TSB (Area Under Curve (AUC) = 0.557) and B/A (AUC = 0.566) was low, indicating that abnormal BAEP should be detected by multiple factors.

Conclusions: Multivariate detection is beneficial for predicting the occurrence of auditory nerve injury in patients with hyperbilirubinemia.

Keywords: neonatal hyperbilirubinemia; risk factors; brainstem auditory evoked potential

Introduction

Neonatal hyperbilirubinemia is a common disease in infants. Severe neonatal hyperbilirubinemia can cause acute bilirubin encephalopathy (ABE), and the most common form of ABE is hearing impairment. This condition affects not only damages the cochlea but also the auditory center of the brainstem [1].

Clinical and epidemiological data show that the incidence of ABE has not decreased year by year. On the contrary, because of the focus on bilirubin levels during clinical diagnosis and treatment [2], the monitoring of risk factors for bilirubin encephalopathy may be neglected, and the lack of specific clinical manifestations in early ABE causes the incidence of ABE to rise slightly over a certain period of time [3]. Bilirubin level is an important risk factor for auditory nerve injury in children since their auditory system is especially sensitive to bilirubin-induced neurotoxicity. It has been shown that neurons in certain regions are susceptible to the toxic effects of bilirubin, among which the most vulnerable is the nucleus of the auditory nerve conduction pathway. Results of previous studies suggest that unbound bilirubin passes through the blood-brain barrier into the cerebrospinal fluid, and can affect the transmission between neurons. Bilirubin interacts with the nerve cell synaptic membrane, affecting synaptic activity. This can prolong nerve conduction, increase intracellular calcium influx, alter the tricarboxylic acid cycle mechanism, and eventually cause hearing impairment. At present, clinical studies support brainstem auditory evoked potential (BAEP) as an objective method for diagnosing hearing impairment. BAEP results can reveal the subcortical struc-

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tural integrity of the auditory pathway and damage at different levels of the brain stem. Waves I and II reflect the state of auditory nerve activity and wave V reflects the auditory activity in the brainstem. Alterations in the measured waves can identify bilirubin-induced hearing impairment in children in the early stages. Current research suggests that auditory neurotoxicity caused by bilirubin is recoverable and that hearing damage is reversible. Thus, it is critical to identify early risk factors for disease as well as additional methods for diagnosis and treatment, since timely detection and intervention may reduce the occurrence of nervous system damage caused by bilirubin. At present, BAEP is the most sensitive method for evaluating bilirubin neurotoxicity [4]. In order to better understand the relationship between BAEP and various risk factors in children with hyperbilirubinemia, this study was conducted in the neonatology department of our hospital from January 2012 to December 2018. 561 neonates with hyperbilirubinemia who underwent BAEP examination were analyzed in groups in order to identify early risk factors for abnormal BAEP results. This research aims to improve early detection and prevention, improve the cure rate of children, and improve the quality of life in the late neonatal period [5].

Materials and Methods

Patients and Definitions

This was a retrospective study of 561 neonates diagnosed with hyperbilirubinemia and evaluated with BAEP at Children's Hospital of Soochow University from January 2012 to December 2018. Exclusion criteria were: (1) Having teratological abnormality or having a family history of permanent deafness; (2) Cytomegalovirus infection, hypoxic ischemic encephalopathy, congenital brain development malformation, etc.; (3) Related genetic metabolic diseases affecting bilirubin levels.

Diagnostic criteria for neonatal hyperbilirubinemia: The level of bilirubin after neonatal birth is a dynamic process, so the diagnosis of hyperbilirubinemia requires consideration of gestational age, post-natal age, and the presence of risk factors. For neonates with gestational age $\geq$35 weeks, the neonatal hour bilirubin line diagram made by Bhutani *et al*. [6], or the American Academy of Pediatrics recommended phototherapy reference curve, are currently used as references for diagnosis or intervention [7].

Diagnosis of bilirubin encephalopathy: severe hyperbilirubinemia and typical neurological symptoms and signs. Symptoms include lethargy, convulsions, abnormal tension of muscle, opisthotonus, etc.; Skull Magnetic Resonance Imaging (MRI) showing symmetrical high signal changes in T1 and T2 weighted images of bilateral globus pallidus [2]; BAEP suggesting high-frequency hearing loss. If the unit does not have a head MRI or BAEP examination, the diagnosis is based on severe hyperbilirubinemia and clinical manifestations.

Data were collected for general characteristics (gender, onset, and admission age, duration of jaundice, gestational age, birth weight), disease during pregnancy (gestational diabetes, gestational hypertension, gestational anemia, pet exposure history, History of high fever infection during pregnancy), birth status (premature rupture of membranes, amniotic fluid contamination, umbilical cord abnormalities, intrauterine distress), total serum bilirubin (TSB), bilirubin/albumin (B/A), routine bloodwork, liver function, toxopasma, rubella virus, cytomegalovirus, herpes simplex virus (TORCH) test, glucose-6-phosphate dehydrogenase (G6PD) activity, blood culture, cerebrospinal fluid examination, etc.), and auxiliary examination (BAEP).

BAEP was performed in all children after 5–7 days of hospitalization using a Danish Madsen brainstem auditory evoked potential meter. Phenobarbital 10 mg/kg was injected intravenously for sedation 30 min before the examination. BAEP anomaly criteria included: the disappearance of one or both sides of I, III, and V waves, partial disappearance of one or both sides of I, III, and V waves, and I, III, and V waves peak latency >normal values of 2.5 s, I~III or III~V interval >normal value 2.5 s, I, III, V wave differentiation abnormalities, ipsilateral III~V >I~III wave interval, V wave response threshold >40 spldb [8].

Statistical Analyses

Statistical analyses were conducted using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Normally distributed variables are expressed as mean $\pm$ standard deviation, non-normally distributed variables are expressed as median (interquartile range), and count data are expressed as percentages. Group differences for normally distributed continuous variables were assessed using the U-test, and differences for non-normally distributed variables were analyzed using the Mann-Whitney U test. Categorical data were analyzed using the chi-squared (χ^2) test or Fisher's exact test. p values < 0.05 were considered statistically significant. A univariate analysis for 11 potential risk factors was performed. The variables identified by univariate analysis ($p < 0.05$) were included in the multivariate regression model to analyze independent risk factors for nerve injury in patients with hyperbilirubinemia.

Results

General Characteristics

A total of 561 children with hyperbilirubinemia who underwent BAEP examination at Children's Hospital of Soochow University between January 2012 and December 2018 were enrolled in the study. The BAEP anomaly group accounted for 198 (35.3%) cases and the BAEP normal group for 363 (64.7%) cases. The difference between the median age of the BAEP anomaly group (7 (5~11) days) and the BAEP normal group (6 (5~10) days) was not statistically significant ($p > 0.05$). However, the difference

Table 1. Comparison of general characteristics between the BAEP anomaly group and the BAEP normal group.

Group	Number	Male	Weight (g)	Cesarean	Age at admission (d)
BAEP normal (-)	363	222 (61.2)	3344.6 ± 412	82 (22.6)	6 (5~10)
BAEP anomaly (+)	198	129 (65.2)	3261.49 ± 512	46 (23.2)	7 (5~11)
Statistics		$\chi^2 = 0.873$	$t = 2.091$	$\chi^2 = 0.030$	Z = –1.958
p		0.35	0.037	0.862	0.05

-, normal; +, anomaly; BAEP, brainstem auditory evoked potential.

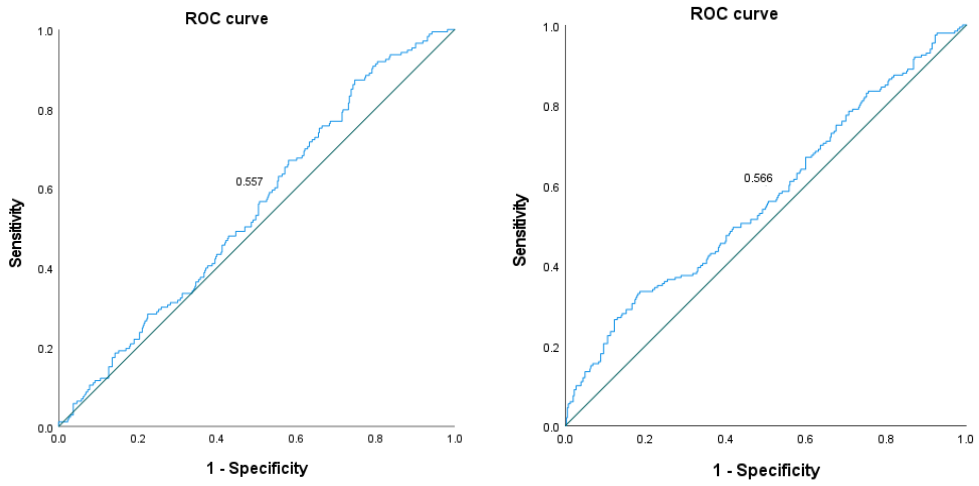

Fig. 1. Accuracy of TSB and B/A in predicting hearing impairment. ROC, receiver operating characteristic.

Table 2. Differences in TSB and B/A values between the two groups.

Group	Number	TSB (mg/dL)	B/A
BAEP (-)	363	373.10 ± 5.07	9.92 ± 1.41
BAEP (+)	198	385.539 ± 68.1	10.39 ± 2.09
Statistics		u = –2.566	u = –2.832
p		0.011	0.005

-, normal; +, anomaly; TSB, total serum bilirubin; B/A, bilirubin/albumin.

in average birth weight between the BAEP anomaly group (3261.49 ± 512) and the BAEP normal group (3344.6 ± 412 g) was significant ($p < 0.05$). There was no significant difference in the percentage of males between the BAEP anomaly group (65.2%, 129 of 198) and the BAEP normal group (61.2%, 222 of 363). Differences in age at admission and percent Caesarean delivery between the two groups were non-significant ($p > 0.05$) (Table 1).

Prediction of BAEP Anomalies by TSB and B/A

Bilirubin-induced neurological dysfunction mainly manifests as severe auditory neuropathy. In clinical work, children with hyperbilirubinemia are often identified by TSB and B/A. Levels of TSB and B/A in the BAEP anomaly group were higher than those of the BAEP normal group ($p < 0.05$) (Table 2). The area under the receiver operating characteristic (ROC) curve for the indicators TSB and B/A were 0.557 and 0.566 (Fig. 1).

Univariate analysis revealed that differences between groups in premature rupture of membranes, gestational age, abnormal umbilical cord, and gestational diabetes during pregnancy were statistically significant ($p < 0.05$) (Table 3).

The statistically significant risk factors identified in univariate analysis were included as independent variables in a multivariate stepwise logistic regression analysis with BAEP anomaly as the dependent variable. The results revealed that gestational age (odds ratio (OR) = 0.82, 95% Confidence Interval (CI): 0.720~0.910), gestational diabetes (OR = 2.715, 95% CI: 1.399~5.269), and premature rupture of membranes (OR = 2.445, 95% CI: 1.211~4.936) were independent risk factors for BAEP anomaly (Table 4).

Discussion

Clinical data in this country and abroad in recent years show that the incidence of ABE has not decreased year by year. Therefore, effective intervention in children with jaundice is critical in order to avoid the occurrence of bilirubin nerve injury and improve the quality of life of newborns. Within the nervous system, bilirubin neurotoxicity is highly selective, and the nucleus of the auditory nerve conduction pathway is especially susceptible [9]. BAEP can record a series of potential responses generated by the entire auditory system, from the auditory nerve to the brainstem to the auditory cortex center, after acoustic stimula-

Table 3. Univariate analysis of risk factors for abnormal BAEP in children with hyperbilirubinemia.

Clinical parameters	BAEP (-)	BAEP (+)	*p-value*
	n = 363	n = 198	
Preterm (n, %)	10 (2.8)	40 (20.2)	<0.001
Umbilical cord (n, %)	10 (2.8)	16 (8.1)	0.040
Amniotic fluid (n, %)	15 (4.1)	12 (6.4)	0.308
Premature rupture of membranes (n, %)	23 (6.3)	22 (11.1)	0.047
Hypertensive disorders (n, %)	9 (2.5)	8 (4.0)	0.313
Gestational diabetes (n, %)	19 (5.2)	24 (12.1)	0.003
Thyroid dysfunction (n, %)	14 (13.9)	2 (1.0)	0.053
History of fever during pregnancy (n, %)	15 (4.1)	7 (3.5)	0.728
Pet contact history (n, %)	4 (1.1)	5 (2.5)	0.200

-, normal; +, anomaly; n, number.

Table 4. Multivariate logistic regression analysis for predictors of BAEP anomaly.

	Wald	*p*	OR (95% CI)
Age	11.5	0.001	0.820 (0.720, 0.910)
Umbilical cord	3.833	0.051	2.326 (0.999, 5.416)
Premature rupture of membranes	6.226	0.013	2.445 (1.211, 4.936)
Gestational diabetes	4.702	0.030	2.715 (1.399, 5.269)

-, normal; +, anomaly; OR, odds ratio; CI, Confidence Interval.

tion. It can fully characterize the functional abnormalities in the neural pathway, can objectively identify the physiological functions and pathological disorders of the brain stem and brain nerve function, and can register abnormalities in the early stage of bilirubin encephalopathy [10]. Critically, early bilirubin neurotoxicity is reversible, which requires us to actively identify whether there are high-risk factors and to find additional means of diagnosis and treatment; timely intervention can reduce the occurrence of bilirubin nerve damage.

The results of this study showed that the TSB and B/A levels in the BAEP abnormal group were significantly higher than those in the BAEP normal group ($p < 0.05$). Furthermore, when the ROC working curve was used to judge the effectiveness of TSB and B/A in predicting hearing impairment, we found that the area under the curve for TSB was 0.557, and for B/A it was 0.566. Thus, the accuracy of diagnosis of hearing impairment was low, which is similar to findings in the previous study [11]. This may be because it is generally believed that serum bilirubin level less than 342 umol/L is a relatively safe range in clinical work; however, for bilirubin levels within this range, although there may be no obvious clinical symptoms, the brain stem auditory evoked potential results may be abnormal. This indicates that even with a bilirubin level that is not particularly high, children can incur neuronal damage, and may suffer nervous system sequelae. In clinical work, the abnormal increases in TSB and B/A are used as an intervention index for hyperbilirubinemia [12]; these measures are considered surrogate parameters for free bilirubin and play a role in the risk assessment of bilirubin encephalopathy. However, they are susceptible to a variety of factors, and clinicians should seek additional indicators for intervention [11].

In this retrospective study, independent risk factors for abnormal BAEP in children with hyperbilirubinemia were premature rupture of membranes, gestational age, and gestational diabetes. Premature rupture of membranes can cause intrauterine infection, and since the fetal immune system is incomplete, sepsis is prone to occur after birth, aggravating bilirubin destruction and bilirubin transformation disorder, as demonstrated in the previous study [13]. For premature infants, the half-life of red blood cells is short and the liver enzyme system is immature; thus, the increase in intestinal liver circulation leads to an increase in bilirubin production. In addition, since the albumin level in premature infants is very low and is susceptible to acidosis, the affinity of unconjugated bilirubin and albumin is closely related to developmental maturity, so premature infants tend to have higher free bilirubin concentrations. In addition, the blood-brain barrier and neurons are immature, the ability of neurons to metabolize bilirubin is poor, and a severe systemic inflammatory response can enhance bilirubin neurotoxicity, which has a greater impact on immature cells and premature infants [3,14], so premature infants can also have bilirubin nerve damage at low levels of free bilirubin, resulting in an increased rate of abnormal BAEP. For premature infants, the clinical manifestations of acute bilirubin encephalopathy are often atypical, difficult to discern, and may co-occur with, and be masked by, other diseases. Abnormalities of the respiratory system (apnea, and decreased oxygen saturation) may be the only clinical man-

ifestations of acute bilirubin encephalopathy in premature infants. Therefore, if the premature infant with jaundice has frequent apnea, the possibility of concurrent ABE should be considered, even if the bilirubin level is not high. For premature jaundice, early intervention and treatment should be prioritized to reduce the occurrence of bilirubin nerve injury-related sequelae in children. Pregnant women with gestational diabetes have long-term high blood sugar levels, which makes the insulin levels in the fetal blood higher; this in turn inhibits the secretion of glucocorticoids, resulting in decreased secretion of active substances by the alveolar epithelial type 2 cells, delaying the maturation of the lungs. If the fetus is in a state of chronic hypoxia in the uterus, it will stimulate the hematopoietic system and increase the amount of red blood cells. After birth, the red blood cells will be destroyed and a large amount of bilirubin will be formed. This is consistent with previous research [15–17]. Abnormal amniotic fluid, especially in neonates with amniotic fluid contamination, leads to early discharge of meconium, which greatly reduces intestinal circulation and reabsorption of bilirubin. An umbilical cord abnormality in the uterus can promote a state of chronic hypoxia and also increase bilirubin production. All of these factors can contribute to high levels of bilirubin in the child, resulting in a high rate of abnormal BAEP.

Conclusions

In summary, further improving the management of children with risk factors through early detection and early intervention, and early prevention and treatment can effectively prevent and avoid the occurrence of neonatal bilirubin nerve injury. The treatment of children with ABE should be proactive, and only in this way can we minimize the occurrence of disability. In addition to strict detection of bilirubin levels, more attention should be paid to the treatment of risk factors, dynamic monitoring of BAEP, and active treatment to effectively reduce the incidence of Bilirubin nerve damage.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding authors upon reasonable request.

Author Contributions

JX and DS were responsible for the design of the study, conduction of the experiment, analysis of the data, and completion of the manuscript. QL, YW and FG were responsible for the data curation, and formal analysis of the study. LL conceived the project, planned and guided the research, and supervised the study. XZ was responsible for the validation and visualization of the study. All authors were involved in the drafting and critical revision of the manuscript. All authors have read and approved the final manuscript. All authors were fully involved in the work and agreed to take responsibility for all aspects of the work.

Ethics Approval and Consent to Participate

All experiments were conducted in compliance with the relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki. The article has obtained ethical approval from the Medical Ethics Committee of Children's Hospital of Soochow University, with ethical number 2024CS029. Informed consent was obtained from all patients before enrollment in the study.

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Conflict of Interest

The authors declare no conflict of interest.

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