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Levels of Neuron-Specific Enolase and S-100B in the Serum of Neonates in Early Diagnosis of Possible Neurotoxic Effects of Hyperbilirubinemia

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Abstract

Aim: The laboratory and imaging methods are not sufficiently sensitive to determine precisely the neurotoxic effects of bilirubin in neonates. The neuron-specific enolase and calcium binding protein B, which are sensitive biomarkers of cellular damage in the central nerve system, were used in the present study to demonstrate possible neurotoxic effects of bilirubin below 20 mg/dL. We hypothesized that neuron-specific enolase and calcium binding protein B might be helpful for our purposes.

Material and Methods: The present study included 33 full-term infants hospitalized for phototherapy treatment (patient group) along with 29 healthy full-term infants (control group). The serum bilirubin levels of the patient group were all below 20 mg/dl. Two serum samples were obtained from all 62 infants at an interval of at least 48hrs which were used for the measurement of bilirubin, calcium binding protein B, and neuron-specific enolase levels.

Results: There was no significant difference in terms of the serum levels of calcium binding protein B between the patient and control groups but there was a significant difference of the serum levels of neuron-specific enolase between the groups. In addition, there were no significant changes in the levels of calcium binding protein B and neuron-specific enolase among the patient group before and after the phototherapy.

Conclusion: We conclude that, considering the serum levels of calcium binding protein B and neuron-specific enolase, a serum bilirubin level of <20mg/dL had no neurotoxic effect on the central nerve system. The results of the present study are consistent with the accepted safe level of bilirubin, <20mg/dL, in a full-term newborn.

Key Words: Bilirubin Encephalopathy; Neonatal Hyperbilirubinemia; Neuron-Specific Enolase; Calcium Binding Protein B.

Hiperbilirubineminin Olası Erken Nörotoksik Etkilerini Belirlemede, Yenidoğanların Serumunda Neuron-Spesifik Enolaz ve S-100B Düzeyler

Özet

Amaç: Yenidoğanlarda bilirubinin nörotoksik etkisini tam olarak göstermede, kullanılan laboratuvar ve görüntüleme metodları yeterince hassas değildir. Çalışmamızda 20mg/dl'in altındaki bilirubinin muhtemel nörotoksik etkisini gösterebilmek için, santral sinir sistemindeki hücresel hasarı gösteren hassas belirteçler olan nöron-spesifik enolaz ve kalsiyum bağlayıcı protein B kullanıldı. Çalışmamız nöron-spesifik enolaz ve kalsiyum bağlayıcı protein B düzeylerini ölçmenin, bu amaç için uygun olabileceği hipotezi üzerine kurgulandı.

Gereç ve Yöntemler: Çalışmada fototerapi tedavisi için hastaneye yatışı yapılan 33 term bebek (hasta gurubu) ve 29 sağlıklı term bebek (kontrol grubu) yer aldı. Fototerapi alması gereken bebeklerin serum bilirubin düzeyleri 20mg/dl'nin altında idi. Bütün bebeklerden, bilirubin, nöron-spesifik enolaz ve kalsiyum bağlayıcı protein B seviyelerini ölçmek için, en az 48 saat arayla iki serum örneği alındı.

Bulgular: Hasta ve kontrol gruplarının kalsiyum bağlayıcı protein B seviyeleri arasında anlamlı bir fark bulunmadı fakat nöron-spesifik enolaz değerleri arasında anlamlı bir fark bulundu. Hasta grubunda fototerapi öncesi ve sonrası nöron-spesifik enolaz ve kalsiyum bağlayıcı protein B değerleri arasında anlamlı bir değişim gözlenmedi.

Sonuç: Kalsiyum bağlayıcı protein B ve nöron-spesifik enolaz serum düzeyleri referans alındığında, 20mg/dl'in altındaki serum bilirubin değerlerinin nörotoksik etkisi olmadığı sonucuna vardık. Bu çalışmanın sonuçları, term bebeklerde kabul edilen güvenli bilirubin seviyesi olan 20mg/dl ile uyumludur.

Anahtar Kelimeler: Bilirubin Ensefalopati; Neonatal Hiperbilirubinemi; Nöron-Spesifik Enolaz; Kalsiyum Bağlayıcı Protein B.

INTRODUCTION

Bilirubin encephalopathy can develop in newborn infants as a result of acute neurotoxic effects of bilirubin. Kernicterus, the most significant chronic complication of neonatal hyperbilirubinemia, is defined as a pathologic change as a result of the accumulation of bilirubin in basal ganglia and brain stem nuclei that can lead to persistent neurological sequelae and even death (1). Bilirubin at concentrations above the exchange transfusion levels has neurotoxic effects. However, neurotoxic effects and long-term minor sequelae related to unconjugated bilirubin below the exchange transfusion levels have not been identified in clinical or laboratory ways (2). The techniques that have been used to demonstrate bilirubin-induced encephalopathy include magnetic resonance imaging (MRI), brainstem auditory evoked potentials (BAEP), oto-acoustic emissions (OAEs), cochlear microphonic (CM) responses,

electroencephalography (EEG), and some biochemical laboratory tests (3,4). All these methods are not able to determine the level of bilirubin that will not cause encephalopathy in infants precisely or the level that will lead to kernicterus in newborns. Biomarkers such as neuron-specific enolase (NSE), Tau protein, calcium-binding protein B (S100B), neurofilament triplet protein, glial fibrillary acidic protein and, brain-specific creatine kinase (CK-BB) can be used to detect the degree of neuronal damage and central nervous system (CNS) pathology. S100B, a member of the S100 family, is produced primarily by astrocytes and is found in other tissues, especially in fat tissues (5,6). Increased levels of S100B after brain injury and ischemia owing to astrocyte damage have been reported and it is suggested that S100B can be used as a biomarker to determine the severity of cellular damage and prognosis (7-9).

Similar to S100B, increased levels of NSE have also been determined following brain trauma and brain pathology. Elevated cerebrospinal fluid (CSF), plasma, and serum levels of both NSE and S100B were reported following several acute neurological diseases (8-11). We hypothesize that NSE and S100B, which have been proven to be sensitive biomarkers for cellular damage of the CNS, might be helpful in determining cellular damages. Thus, the present study has been designed to show bilirubin neurotoxicity, if any, by measuring serum levels of NSE and S100B in newborns requiring phototherapy. We have investigated the effect of phototherapy on the levels of these two neuron-specific proteins.

MATERIAL AND METHODS

The present study included 62 full-term newborn infants with postnatal ages in the range of 2–20 days. The patient group consisted of 33 jaundiced newborns hospitalized for phototherapy and the control group consisted of 29 healthy full-term newborns from the post-delivery clinic or outpatient healthy infant clinic. All infants were born at a gestational age of at least 37 completed weeks and within appropriate for gestational ages. The inclusion criteria for the patient group contained the presence of hyperbilirubinemia with a total level of serum bilirubin (TSB) <20mg/dL. Infants with any other health problems were excluded. We have obtained the consent from the parents of all infants that were included in the study. The study was approved by the ethical committee of our hospital. The mean birthweight of the newborns was 2998 (±373) g in the patient group, and 3014 (±382) g in the control group. There were 18 males and 15 females in the patient group and 21 males and 8 females in the control group. In the patient group, 12 infants were born via caesarean section and 21 infants were born by spontaneous vaginal delivery. In the control group, 13 infants were born via caesarean section and 16 patients were born by spontaneous vaginal delivery. The Apgar score of all infants was ≥8 at 5 mins after birth.

We have used the phototherapy schedule recommended by the American Academy of Pediatrics to identify the infants requiring phototherapy (2). Serum samples of the patient group were obtained before phototherapy for the measurement of TSB, S100B and NSE. The serum levels S-100B and NSE were measured by an electrochemiluminescence device (Modüler E-170 Roche-Hitachi®, Tokyo, Japan). During the phototherapy sessions, the eyes of the infants were shielded and the genital areas were covered. A fluorescent lamp emitting blue light at wavelength 420–470nm was used as the light source and the distance between the phototherapy lamps and the infant was 30–40 cm. A second serum sample was collected from each infant in the patient group after the phototherapy, which at least lasted for 48 hrs. Baseline serum samples were collected from the control group of infants at the beginning of the study and again after ≥48hrs for the measurement of bilirubin, S100B, and NSE.

Statistical analysis were carried out with the Statistical Package for Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA). The results were expressed in percentages for categorical variables. Continuous variables were presented as mean± standard deviation or median [min – max] as appropriates. The χ^2 test was used to determine the relationship between categorical variables. Independent samples *t*-test was used for comparing the two groups if parametric test assumptions were satisfactory. If these assumptions were not satisfactory, Mann Whitney U test was preferred. Within the groups, differences were given by paired samples t test. Factors affecting the delta-S100 B and delta-NSE were analyzed by multiple linear regression analysis. The level of statistically significant difference was set at $p \leq 0.05$.

RESULTS

There was no significant difference between the groups with respecs to gender and mode of birth; but there was significant differences between birth weights (Table 1).

Table 1. The general characteristics of the infants

	Patient Group (n=33)	Control Group(n=29)	p
Gender, n (%)			
Male	18 (54.5)	21 (72.4)	0.234
Female	15 (45.5)	8 (27.6)	
Mode of birth, n (%)			
Vaginal	21 (63.6)	16 (55.2)	0.676
C/S	12 (36.4)	13 (44.8)	
Birth weight (g), mean±SD	2998±373	3456±456	<0.001

C/S: Cesarean section, SD: Standard deviation

The mean level of TSB in the patient group was 19.5 (±2.80) mg/dL before phototherapy and each infant in this group received phototherapy according to the recommended schedule. At baseline, the mean level of TSB in the control group was 2.5 (±2.00) mg/dL and no infant in this group required phototherapy according to the recommended phototherapy regulations.

At baseline, the mean level of S100B was 1.5 (±0.55) µg/L in the patient group and 1.6 (±0.51) µg/L in the control group. The mean level of NSE was 43.5 (±16.20) ng/mL in the patient group and 63.1(±31.20) ng/mL in the control group; but there was a significant difference between the two groups in terms of NSE levels at baseline and after 48 hours (Table 2).

Table 2. The comparison of patient and control groups in terms of TSB, S100B and NSE levels at baseline and after 48 hours

	Patient Group (n=33)	Control Group (n=29)	p
TSB levels (mg/dL), mean±SD			
At baseline	19.50±2.80	2.50±2.00	<0.001
After 48 hours	11.40±2.70	8.40±1.90	<0.001
S100B levels (µg/L), mean±SD			
At baseline	1.56±0.55	1.62±0.51	0.654
After 48 hours	1.70±0.62	1.43±0.43	0.055
NSE levels (ng/ml), mean±SD			
At baseline	43.5±16.2	63.1± 31	0.003
After 48 hours	41.4±11.0	59.7± 23	<0.001

TSB: Total serum bilirubin, NSE: Neuron specific enolase, SD: Standard deviation

Phototherapy was continued in the patient group for at least 48 h. The levels of TSB, S100B and NSE were measured after phototherapy. In the patient group, the mean levels of bilirubin, S100B and NSE were 11.4 (±2.70) mg/dL, 1.7 (±0.62) µg/L and 41.3 (±10.99) ng/mL, respectively, after 48 hrs of phototherapy. No significant difference was found in the levels of S100B and NSE before or after phototherapy in the patient group (Table 3). So, there was no correlation between the level of TSB and that of S100B or NSE (Table 4).

Table 3. The comparison of mean S100B and NSE levels of patient group before and after phototherapy

	Before Phototherapy	After Phototherapy	p
S100B (µg/L), mean±SD	1.56±0.55	1.70±0.62	0.362
NSE (ng/ml), mean±SD	43.53±16.20	41.37±10.99	0.423

NSE: Neuron specific enolase, SD: Standard deviation

Table 4. Multiple Linear Regression Analyses Results

		Beta* (95 % CI)	p-value
Delta S100 B	Constant	0,027 (-0,490 – 0,543)	0,918
	Patient Group	0,470 (-0,610 – 1,550)	0,387
	Delta TSB	0,013 (-0,058 – 0,085)	0,711
Delta NSE	Constant	4,377 (3,885 – 4,868)	<0,001
	Patient Group	0,380 (-0,649 – 1,408)	0,463
	Delta TSB	0,008 (-0,060 – 0,076)	0,805

* Coefficient of Regression, CI: Confidence Interval. p<0.05

In the control group, the mean level of bilirubin, S100B, and NSE after 48 hrs was 8.4 (±1.90) mg/dL, 1.4 (±0.43) µg/L, and 59.7 (±23.62) ng/mL, respectively, and there was no significant difference between the level of S100B and NSE before and after 48 hrs (Table 5).

Table 5. Comparison of the S100B and NSE levels of the control group

	At baseline	After 48 hours	p
S100B (µg/L), mean±SD	1.62±0.51	1.43±0.43	0.091
NSE (ng/ml), mean±SD	63.14±31.20	59.75±23.62	0.671

NSE: Neuron specific enolase, SD: Standard deviation

DISCUSSION

It is a common problem in newborns that hyperbilirubinemia can lead to serious neurological dysfunctions if treatment is inadequate or belated. Current clinical, laboratory, and imaging methods are not able to determine the precise level of bilirubin that will not cause encephalopathy in infants or the level that will lead to kernicterus in newborns (2). The use of neuron-specific biomarkers, such as NSE, Tau protein, S100B, neurofilament triplet protein, glial fibrillary acidic

protein, and CK-BB might be helpful for developing APT methods to demonstrate the neurotoxic effects of hyperbilirubinemia.

The calcium-binding protein S100B is a member of the S100 family that can be isolated from the brain tissue, where it functions as a neurotrophic factor and a neuronal developmental protein. Levels of S100B increase in patients with head injury and CNS pathology due to astrocyte damage (12-14). The serum level of S100B is higher in newborns with hypoxic ischemic encephalopathy (HIE) and it is reported that newborns with serum levels of S100B >12 µg/L frequently have fatal outcomes or develop cerebral palsy (15). S100B can be measured in CSF, urine, and serum (16). The serum levels of S100B can be high in preterm infants with intracranial hemorrhage or in newborns with HIE even in the absence of any other clinical, laboratory or ultrasonography findings (17,18).

NSE is a glycolytic dimeric enzyme found in neuronal and neuroendocrine cells of the central and peripheral nervous systems and constitutes 0.4–4% of the soluble proteins of the brain. Brain-specific NSE is an isoenzyme of the enolase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway. Increased levels of NSE have been determined in the CSF and serum of full-term infants with asphyxia and cerebral infarcts (19,20).

Infants with moderate hyperbilirubinemia can display neurological problems and developmental retardation. Transient changes in behavior and crying patterns have been demonstrated by brainstem auditory evoked response recordings in infants with bilirubin levels of 15–25 mg/dL (1). There are many reports that relate neurotoxic effects in full-term infants with TSB levels >20 mg/dL (21-23).

In the present study, we used S100B and NSE, which are sensitive biomarkers of neuronal damage, to demonstrate whether serum levels of TSB <20 mg/dL are safe for infants. There are few reports that associate S100B and NSE levels with hyperbilirubinemia. In their study investigating the serum level of NSE in a rat model of kernicterus, Semba and Kato have reported a three-fold higher plasma level of NSE and a >30-fold higher level of NSE in the CSF in the rat model controls (24). Akman *et al.* reported that the serum level of NSE has increased significantly in infants with auditory neuropathy due to hyperbilirubinemia, despite the lack of a significant association between bilirubin and the serum level of NSE (25). Okumus *et al.* have reported a significant increase in the serum levels of Tau and S100B in correlation with serum levels of TSB >19.1 mg/Dl (26).

We found no significant difference in terms of serum levels of S100B between the patients in the study and control groups. We found, however, a significant difference in the serum levels of NSE between the two groups. Meanwhile there was also significant difference between the groups with respect to birth weights. NSE is found in mature neurons and cells of neuronal origin. We did not study the gestational ages of the infants but the statistical difference of the serum leves of NSE can be attributed to immaturity of the infant brains in the patient group.

There was no significant change in the serum levels of S100B and NSE in the patient group before and after phototherapy. Thus, total bilirubin levels <20 mg/dL can be considered to be non-neurotoxic. There was no significant decrease in the serum levels of S-100B and NSE in the patient group after phototherapy; therefore, we concluded that there was no positive effect of phototherapy on the level of these neuron-specific proteins. In the present study, the mean serum level of TSB in infants receiving phototherapy was <20 mg/dL 19.5(±2.80) mg/dL, which might explain why we did not observe increased levels of NSE or S100B proteins.

CONCLUSIONS

Although the NSE and S100B proteins are sensitive to neuronal damage, assessment solely based on these proteins cannot eliminate the presence of bilirubin neurotoxicity. Thus, other imaging and laboratory methods are needed to demonstrate bilirubin-induced encephalopathy while studies with larger numbers of infants should be undertaken to resolve this issue.

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