Very high hyperbilirubinaemia in neonates to identify brain injury, neuron-specific enolase, calcium binding protein B, glial fibrillary acidic protein, Tau protein and growth differentiation factor 5 levels

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Research Article

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Abstract

Objectives: A growing body of evidence suggests blood biomarker analysis may be a useful tool to aid in the elucidation of important pathophysiological mechanisms across a range of severities in newborn TBI. The aim of this research was to use blood biomarkers (NSE, s100B, GFAP, Tau and GDF-5) as a tool to advance knowledge of very high hyperbilirubinaemia in neonates to identify brain injury processes across the severity spectrum of TBI.

Material and Methods: In this prospective study, newborns with bilirubin levels high enough to require exchange transfusion were evaluated for brain damage using GDF-5 level profiles in the laboratory of the Dicle University Faculty of Medicine between August 2016 and August 2017. On the follow-up form, demographic information as well as clinical and laboratory results for the newborns who made up the study's sample were recorded.

Results: The study's results showed that among the serum proteins examined upon the arrival of patients in the experimental group, GFAP, NSE, and s100B were statistically significantly higher in the experimental group than in the control group. Despite the patient group's high GDF5 and MAPt values, they did not statistically significantly.

Conclusion: In conclusion, our study revealed a rise in serum NSE and GFAP levels upon admission and on the third day in the extremely high hyperbilirubinemia newborn experimental group. In addition, neonates in the control group had significantly elevated s100B levels on the day of admission, but not on the third day. In addition, our data imply that NSE and GFAP may be a viable, possible biomarker for extremely high hyperbilirubinaemia in newborns that merits further investigation.

Introduction

Neonatal jaundice is the leading reason for hospitalization in the first week of life [1–2]. Severe hyperbilirubinemia, which can cause kernicterus and neurodevelopmental issues, affects fewer than 2 percent of newborns [3]; however, jaundice affects between 60 and 80 percent of newborns. Bilirubin is typically stored in the brain's basal ganglia, and it affects the central and peripheral auditory and visual pathways, hippocampus, diencephalon, subthalamic nuclei, midbrain, cerebellum and cerebellar vermis regions, and pontine nucleus, as well as portions of the brain stem involved in oculomotor function, respiration, neurohumoral and electrolyte control. [4].

In some developing nations, the incidence of severe neonatal jaundice is approximately 100 times higher than in developed nations; acute bilirubin encephalopathy has been reported in 3 percent of hospitalized infants with jaundice [5]. In population-based studies, the incidence of kernicterus in Denmark, Sweden, and Canada was between 1.2 and 2.3 per 100,000 live births per year, while it was 0.44 in the United States [6]. 6.4% of 5620 newborns hospitalized for jaundice in Turkey were found to have severe hyperbilirubinemia (sTB25mg/dl), and 0.2% had ABE [7]. Severe hyperbilirubinemia is defined as a serum TB level of > 20 mg/dl, > 25 mg/dl, or high enough to require exchange transfusion; sTB > 30 mg/dl is

considered severe hyperbilirubinemia [8]. Kernicterus develops if ABE is not diagnosed and treated early. It is known that the risk and severity of neurotoxicity increase as the serum bilirubin level rises; however, it is unknown at what serum bilirubin level the neurotoxic effects begin to manifest.

Focusing on the identification and treatment of infants at risk of developing severe hyperbilirubinemia, the APA developed a clinical practice guideline for infants born 35 weeks in 2004; the guideline was revised in 2009 by the addition of clinical and laboratory risk factors [9]. Taking into account laboratory and clinical factors, the most recent version of the APA identified the following risk factors for severe hyperbilirubinemia and neurotoxicity: immune hemolytic disease, G6PD deficiency, asphyxia, sepsis, presence of acidosis, and albumin 3 g/dl [10]. In the 2004 APA guideline, it was stated that the sTB/Alb ratio could not be used to make a treatment decision on its own [11, 12].

Biomarkers have a wide range of applications, including diagnosing the presence or absence of pathology, staging and classifying severity, indicating prognosis, and predicting and monitoring treatment responses [13]. Damage to parenchymal structures such as neurons and astroglial cells causes the immediate extracellular release of injury-related molecules, such as calcium binding protein B (s100B), glial fibrillary acidic protein (GFAP), and neuron specific enolase (NSE). A growing body of evidence suggests, however, that blood biomarker analysis may be a useful tool for elucidating important pathophysiological mechanisms across a spectrum of TBI severity. Recent evidence suggests that tau measured peripherally in the systemic circulation may be indicative of CNS tauopathy [14, 15] or axonal damage [16].

NSE, calcium-binding protein B (s100B), Glial Fibrillary Acidic Protein (GFAP), and Tau protein are indicators of cellular damage in the central nervous system, particularly when bilirubin levels are elevated enough to require exchange transfusion and in recent studies on neuronal damage. By measuring the growth differentiation factor 5 (GDF-5) levels, which have begun to be utilized, it is intended to assess the diagnostic sensitivity of these markers. The aim of this research was to use blood biomarkers (NSE, s100B, GFAP, Tau and GDF-5) as a tool to advance knowledge of very high hyperbilirubinaemia in neonates to identify brain injury processes across the severity spectrum of TBI.

Material And Methods

In this prospective study, newborns with bilirubin levels high enough to require exchange transfusion were evaluated for brain damage using GDF-5 level profiles in the laboratory of the Dicle University Faculty of Medicine between Month 202X and Month 2020X. On the follow-up form, demographic information as well as clinical and laboratory results for the newborns who made up the study's sample were recorded. SE, OO, and HY the principal investigators, conducted these follow-ups.

The Dicle University Medical Faculty Hospital Ethics Committee, with reference number 2014-65, granted approval for the study. The Helsinki Declaration Criteria were also followed in protecting the patients' identities and medical information.

43 newborns (Experimental Group, EG: 23; Control Group,CG: 20) who were chosen in accordance with the research sample's inclusion criteria make up the study's sample. From this sample, 20 healthy newborns from the postnatal clinic or outpatient clinic made up the control group, while the experimental group included 23 newborns with jaundice, 23 of whom were hospitalized for phototherapy.

Patient inclusion criteria for the study:

- Newborns older than 24 hours,
- Babies born on time,
- Babies whose indirect bilirubin level is above the blood exchange limit for age and weight.

Patient exclusion criteria from the study:

- Babies in the first 24 hours,
- Babies who are thought to have hydrops fetalis,
- Babies with suspected TORCH group infection,
- Babies with high direct bilirubin levels,
- Babies with a history of perinatal asphyxia,
- Patients with suspected clinical or laboratory sepsis,
- Patients with a preliminary diagnosis of metabolic disease,
- Premature newborns,
- Those born under 2500 grams,
- Babies of diabetic mothers,
- Those with congenital heart disease,
- Patients with suspected hemoglobinopathy,
- Newborns with dehydration,
- Patients diagnosed with congenital hypothyroidism,
- Patients with renal failure,
- Patients with pathology requiring surgical intervention,
- Patients with intracranial bleeding,
- Patients with genetic disease (trisomy, etc.).

Collection of blood samples. American Pediatrics Academy (APA) recommended phototherapy schedule was used to identify infants in the patient group who required phototherapy [17]. Before phototherapy, TSB, GDF5, MAPt, GFAP, S100B, and NSE concentrations were measured in newborn serum samples from the patient group. S-100B and NSE serum levels were measured using an electrochemiluminescence device. On the third and seventh day after at least 48 hours of phototherapy, serum samples were collected from each infant in the patient group. The biochemistry laboratory measured the levels of

bilirubin, TSB, GDF5, MAPt, GFAP, S100B, and NSE in infants' sera using the Enzyme-Linked Immunosorbent Assay (ELISA) method upon the arrival of the control group. After centrifuging blood samples at 6000 rpm for 10 minutes, the separated plasma samples were transferred to an Eppendorf tube and stored at -80 degrees until analysis. Blood samples were delivered to the Biochemistry Department Laboratory at Dicle University using dry ice.

Statistical analysis. Using the IBM SPSS 22.0 Version program, statistical analysis of the research data was conducted. Using the Chi-square Test, the relationship between categorical characteristics and groups was investigated. Meanstandard deviation and median are provided as descriptive statistics for numerical variables, while number and percentage values are provided for categorical variables. The significance level of p0.05 was accepted in statistical analysis. If the parametric test assumptions were met, the two groups were compared using a t-test on independent samples. If these assumptions are not acceptable, the Mann Whitney U test is recommended. The paired samples t-test was used to determine group differences. The level of statistical significance was determined to be p 0.05.

Results

Table 1 lists the demographic distribution of the newborns who participated in the study.

Table 1
shows the patient and control groups' demographic details.

Demographic Features		Experimental Group (EG) (n: 23)	Control Group (CG) (n: 20)	p
Gender	Male	12	12	0.606
	Female	11	8	
Postnatal Age (Days)		5.0(1–14)	5.0(2–11)	0.348
Gestational Age (Weeks)		37.3 ± 2.42	37.8 ± 2.43	0.503
Birth Weight		2744 ± 585	2851 ± 342	0.476

According to Table 1, there is no statistically significant difference between the groups for gender and birth weight of neonates with demographic features.

Statistical characteristics. 10 patients in the experimental group were A Rh+, 2 patients were A Rh-, 7 patients were B Rh+, and 4 patients were O Rh+. 3 patients were A Rh+, 2 patients were A Rh-, 3 patients were B Rh+, 11 patients were O Rh+, 2 patients were O Rh-, and 2 patients were AB Rh-.

Etiological evaluation. While isolated ABO incompatibility was detected in 7 of 23 patients in the experimental group, isolated Rh incompatibility was observed in 4 patients, and isolated subgroup

incompatibility was observed in 1 patient, combined ABO and subgroup incompatibility was observed in 3 individuals. Four patients exhibited urinary tract infection, one patient had omphalitis, and five patients exhibited dehydration.

Table 2 provides a comparison of the experimental and control groups in terms of laboratory parameters.

Table 2
Laboratory parameter comparison between the patient and control groups

Laboratory Parameters	EG (n: 23)	CG (n: 20)	p
TSB (Total Serum Biluribini)	20.75 ± 6.33	4.55 ± 2.05	p < 0.001
DB (Direct Bilirubin)	0.74 ± 0.26	0.60 ± 0.18	0.068
WBC (White Blood Cell)	17.86 ± 8.24	16.38 ± 5.92	0.510
HCT (Hematocrit)	48.17 ± 9.27	49.64 ± 5.95	0.545
CRP (C-Reactive Protein)	0.01(0.01–1.52)	0.06(0.01–0.40)	0.273
TSH (Thyroid Stimulating Hormone)	5.07(0.56–32.18)	4.96(0.56–13.69)	0.652
T4 (Thyroxine)	21.93 ± 4.43	22.48 ± 3.97	0.672

Laboratory parameter comparison between the patient and control groups (Table 2).

In terms of DB, WBC, HCT, CRP, TSH, and T4, Table 2 reveals no significant differences between the experimental and control groups. In comparison to the control group, the experimental group exhibited statistically significant increases in total bilirubin levels. Before phototherapy, the mean TSB level in the experimental group was 20.75 (6.33) mg/dL, and each infant in this group received phototherapy per the recommended protocol. The mean TSB concentration in the control group at baseline was 4.55 (2.05) mg/dL, and no infant in this group required phototherapy according to the recommended phototherapy protocols.

The serum protein levels of the experimental and control groups are compared in Table 3.

Table 3
Comparison of serum protein levels between patient and control groups

Levels of Protein	EG (n: 23)	CG (n: 20)	p
GDF5 (Growth Diferentiation Factor 5)	3.58(1.38–14.23)	2.98(1.18–6.37)	0.082
MAPt (Microtubule Associated Tau Protein)	4011(1540–15996)	3336(1311–7148)	0.084
GFAP (Glial Fibrillary Acidic Protein)	1.44(0.33–9.22)	0.83(0.35–2.76)	0.005
NSE (Neuron Specific Enolase)	8.10(0.91–56.02)	3.66(0.02–9.34)	0.010
s100B (calcium-binding protein B)	32.17(14.51–373.0)	19.71(9.37–84.53)	0.013

According to Table 3, GFAP, NSE, and s100B were statistically significantly greater in the experimental group than in the control group among the serum proteins analyzed upon the arrival of patients in the experimental group ($p < 0.05$). Despite the fact that GDF5 and MAPt values in the patient group were high, they were not statistically significant (p: 0.082–0.084, respectively).

Figure 1 depicts the comparison of NSE, GFAP, and S100P levels on the first and third days between the control group and the experimental group.

Figure 1 demonstrates that the NSE and GFAP levels of the experimental group at admission and on the third day were statistically substantially higher than those of the control group (p0.05). While s100B levels were significantly different between control and admission ($p = 0.013$), there was no link between control and the third day ($p = 0.054$).

Discussion And Conclusion

Due to its lipophilicity, bilirubin, which readily binds to membrane lipids and rapidly crosses the blood-brain barrier (KBB), has a deleterious effect on the brain at high serum concentrations [1, 4]. It is known that the risk and severity of neurotoxicity increase as the blood bilirubin level rises; however, it is unknown at what serum bilirubin level the neurotoxic consequences begin to manifest. APA (2004) reported that infants discharged after 72 hours reduced the incidence of neurotoxicity [12].

According to studies [18, 19], abnormal weight loss is a risk factor for newborn jaundice. Pathological weight loss reflects the infant's feeding problems; the risk of severe hyperbilirubinemia is elevated in infants who are not nursed often and, as a result, do not consume sufficient calories. Salas et al. discovered that pathological weight loss in breastfed newborns nearly quadrupled the probability of non-hemolytic severe hyperbilirubinemia [20]. According to Erdeve et alstudy, .'s weight loss of more than 10 percent was observed in 12,4 percent of hospitalized babies with jaundice, and it was determined to be the second most important risk factor for severe hyperbilirubinemia [18]. In our investigation, however, no statistically significant differences in birth weights of newborns with severe hyperbilirubinemia were detected.

In the 2004 APA guideline, jaundice in the first 24 hours and a history of phototherapy in an older sibling were identified as substantial risk factors for severe hyperbilirubinemia [12]. Independent of other risk factors, the presence of a sibling with jaundice and a sibling with severe hyperbilirubinemia increased the risk of jaundice by 3.1 times and the risk of severe hyperbilirubinemia by 12.5 times in a study of 3301 newborns in the United States. The role of genetic factors in the recurrence of hyperbilirubinemia has been emphasized [21]. In the study, the total bilirubin levels of the unwell infants in the experimental group were significantly higher than those of the infants in the control group. Therefore, each infant in the experimental group received phototherapy according to the suggested protocol.

Understanding the relationship between blood biomarkers and injury-induced brain processes is crucial for advancing our pathophysiology understanding of secondary injury in people. Concerns about peripheral indicators of CNS processes include 1) how blood biomarkers mechanically link to brain injury/repair signaling and 2) the potential extracranial sources, if any, that could confound such markers. Recent evidence suggests that in the acute phase following TBI, brain-derived biomarkers enter the circulation via glympathic clearance, and that disruption of glympathic function may impede the clearance of such molecules into the circulation, thereby making peripheral biomarker detection after injury difficult [22–24].

However, disruption of the blood-brain barrier (BBB) and neuroendocrine dysfunction may affect peripheral biomarker levels following brain injury [25]. Indeed, changes in hormones produced by both the sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA)-axis can modulate peripheral immune function, and Merchant-Borna et al. [26] observed that 7 days after SRC, gene transcription profiles in peripheral blood leukocytes reflected regulation of the HPA-axis. In addition, we recently established [25] that peripheral catecholamine release is substantially linked with circulating levels of inflammatory cytokines and chemokines following moderate and severe TBI. The activation of neural afferent and efferent pathways can inhibit cytokine production via cholinergic signaling, a mechanism that has been shown to specifically inhibit the production of TNF-a by macrophages [27].

The mechanical trauma that causes traumatic brain injury may damage parenchymal tissue, resulting in the release and transit of certain molecules into the bloodstream. Nonetheless, the examination of CNS damage indicators in peripheral blood after TBI has resulted in a number of significant contributions. s100B is the most researched biomarker in traumatic brain injury [28]. Numerous investigations have demonstrated immediate blood increases in individuals with all types of injuries [29, 30]. GFAP is the primary intermediate filament protein in astrocytes, and, like s100B, it has been investigated extensively across the spectrum of TBI. It is believed that reactive astrocyte gliosis or astrocyte damage leads to the release of GFAP from the CNS following injury [31]. Indeed, acutely high blood concentrations of the protein have been reported across the severity spectrum of TBI [32] and are associated with poor patient prognosis [33]. GFAP may be superior than s100B as a TBI biomarker due to its increased brain specificity, although this is not conclusive. It outperforms s100B in diagnostic sensitivity and specificity in severe injuries and is unaffected by extracranial damage [30]. In the limited number of studies evaluating GFAP-BDP in TBI patients, positive associations with damage severity, structural abnormalities, and the

requirement for neurosurgical intervention have been reported [32]. Neuron specific enolase (NSE) is an enzyme involved in glycolysis that is primarily neuronal. Increased blood concentrations have been seen in patients with mild and severe TBI and have been associated with a poor prognosis [33]. NSE's presence in erythrocytes and vulnerability to post-processing hemolysis and extracranial damage [34] have reduced its utility as a biomarker for traumatic brain injury. In addition, comparable to s100B, acute increases in circulating NSE levels have been observed after physical activity [30, 32]. Mutations in the MAPT gene are known to produce tauopathy and are related with the development of neurodegenerative disorders [35]. The blood proteins GFAP, NSE, and S100 were statistically substantially greater in the experimental group than in the control group, as determined by the research findings. Despite the fact that the GDF5 and MAPt values in the patient group were high, they were not statistically significant. This is due to the number of patients in the experimental group.

Limitations of the present study include the small sample size, and the diagnostic significance of blood parameters in newborns with extremely high hyperbilirubinemia should be assessed in future studies with larger sample sizes. In conclusion, our study revealed a rise in serum NSE and GFAP levels upon admission and on the third day in the extremely high hyperbilirubinemia newborn experimental group. In addition, neonates in the control group had significantly elevated s100B levels on the day of admission, but not on the third day. In addition, our data imply that NSE and GFAP may be a viable, possible biomarker for extremely high hyperbilirubinaemia in newborns that merits further investigation.

Declarations

For the study, ethic approval has been taken from Dicle Univercity Medical Ethics Comittee For Noninterventıonal Studies at 25.11.2014.

Informed concent has been taken from all the participants for both inclusion in the study and publication.

Data has been taken from the patient files.

All authors declare that there is not any financial or non-financial competing interests.

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All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Özhan Orhan, Sabahattin Ertuğrul and Hatice Yüksel. The first draft of manuscript was written by Özhan Orhan and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

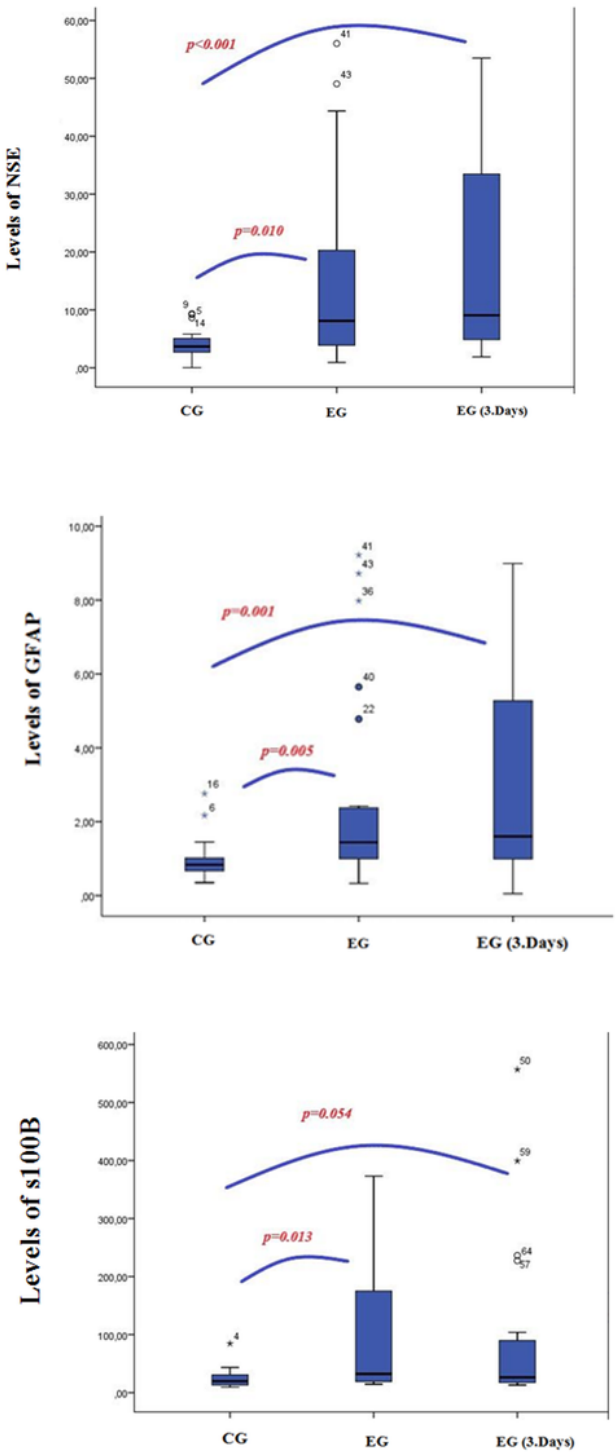

Figure 1

Comparison of NSE, GFAP, and S100P levels between the control group and the experimental group on the first and third days