ORIGINAL ARTICLE

Evaluation of serum brain-derived neurotrophic factor levels in preterm and term neonates and its association with hyperbilirubinemia

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Abstract

Background: Neonatal hyperbilirubinemia is the most common cause of hospital admission in the first month of life. Maternal, neonatal, and prenatal factors affect the severity of neonatal hyperbilirubinemia. It has been reported that with increase in bilirubin levels, there is a risk of neuronal cell damage. Brain-Derived Neurotrophic Factor (BDNF), a neurotropic factor produced in the brain, is an important tool to assess the neuronal activity in the neonates. *Aim and Objectives*: This study aims at evaluating serum BDNF and bilirubin levels among neonates with clinically diagnosed preterm hyperbilirubinemia, term hyperbilirubinemia and full term normal healthy neonates and correlating serum BDNF levels with serum total bilirubin levels in these group of neonates. *Material and Methods:*This is a cross-sectional study, which was done in left over blood samples from hyperbilirubinemic neonates in clinical biochemistry laboratory, Kasturba Medical College Hospital, over a period of 4 months.Total number of 90 subjects were included in the study. Normal healthy full-term neonates (gestational age ⩾ 38 weeks; Group I), pre-term neonates (gestational age ≥ 31-37 weeks; Group II) with clinically diagnosed hyperbilirubinemia and term neonates (gestational age ≥ 38 weeks; Group III) with clinically diagnosed hyperbilirubinemia were included. *Results:* The current study showed significant decrease in serum levels of BDNF in preterm and term neonates with hyperbilirubinemia when compared to control ($p<0.05$). There was a negative correlation of serum BDNF levels with bilirubin levels across all groups. *Conclusion:* This study may be useful in performing neurological risk assessment in full term and preterm neonates with hyperbilirubinemia which may aid in clinical decision making.

Key words: BDNF, Hyperbilirubinemia, Preterm neonates

Introduction

Neonatal hyperbilirubinemia, which is due to a physiological imbalance between the synthesis and elimination of bilirubin, occurs in most newborn infants. It is the most common cause of hospital admission in the first month of life [1]. A serum total bilirubin level above 5 mg/dl in neonates is suggestive of neonatal jaundice. In first week of newborn life around 60% of term and 80% of pre-term babies develop jaundice, and at 1 month about 10% of breastfed babies are still jaundiced. This altered condition is associated with variety of biochemical imbalances [2].

Physiological jaundice of the newborn which is due to the deficiency in bilirubin glucuronyl

transferase enzyme could be life threatening [3]. In certain cases, elevated circulating bilirubin levels can result in severe neonatal hyperbilirubinemia (if left untreated) resulting in Acute Bilirubin Encephalopathy (ABE), Chronic Bilirubin Encephalopathy (CBE), kernicterus, and permanent neurologic disorder characterized by dystonia, choreoathetosis, hearing loss, and oculomotor pareses produced by neuropathological damage to the basal ganglia, hippocampus, cerebellum, and brainstem [4]. During embryonal development, neuronal death occurs only by apoptosis and not by necrosis. Apoptotic neuronal damage may be responsible for altered brain development which will be associated with prematurity and perinatal abnormality. Neurotrophic factors play important roles in neuro protection. Nerve growth factors like brain-derived neurotrophic factor, and neurotrophin 3 are neurotrophins that act on Tyrosine kinase (Trk) A, Trk B and Trk C receptors, respectively. Neurotrophic factors such as Brain-Derived Neurotrophic Factor (BDNF) and NT3 play important roles in protecting neurons from entering or progressing along an apoptotic pathway [5-6]. They play an important role in growth, differentiation, development and survival of neurons. Neurotrophic factors have long been implicated in neuronal survival, cortical development, and synaptic plasticity. In addition to anti-apoptotic activities, neurotrophins play crucial roles in axon growth during development, morphologic differentiation, neurotransmitter expression and higher neuronal functions [7-8]. Thus, neurotrophins may play crucial roles in antenatal and post-natal brain development.

However, studies regarding the presence and effect of neurotrophins like BDNF in premature and mature newborn neonates are insufficient. Although not established, it is plausible that altered blood levels of BDNF status would contribute to morbidity and mortality among hyperbilirubinemic newborns. The incidence of abnormal BDNF status in hyperbilirubinemia patients has not been comprehensively studied. Therefore, this study aims to estimate and correlate serum BDNF levels in clinically diagnosed preterm, term hyperbilirubinemic neonates and full term normal healthy newborn controls.

Material and Methods

It was a cross-sectional study, which was done in blood samples from hyperbilirubinemic neonates in Clinical Biochemistry Laboratory, Kasturba Medical College Hospital, Attavar, Mangalore for a duration of 4 months. Total number of 90 subjects were included in the study.

(Sample size was achieved by using the sample size calculator for the power of > 90% and alpha value < 0.05) [9].

Sample size:

$$n = \frac{2(z\alpha+z\beta)^2 * \sigma^2}{d^2}$$

$Z_\alpha = 1.96$

$Z_\beta = 1.96$

d =mean difference between groups.

Sample size calculated (n) =30

Study was performed after obtaining ethics approval by Institutional Ethics Committee and informed consent was obtained from all parents of neonates included in the study.

Inclusion criteria

Group 1: Thirty (control) full term healthy newborn (gestational age ⩾ 38 weeks) with serum bilirubin values within normal range.

Group 2: Thirty preterm newborns (gestational age 31- 37 weeks) with serum bilirubin values (⩾ 8 mg/dl)in the treatment threshold.

Group 3: Thirty full term newborns (gestational age ⩾ 38 weeks) with serum bilirubin values (⩾ 10mg/dl) in the treatment threshold.

Exclusion criteria

New-born babies with severe sepsis, congenital hepatobiliary malformations, congenital cholestatic jaundice, and birth asphyxia were excluded.

Collection of samples

Blood samples from preterm newborns with hyperbilirubinemia, full term newborns with hyperbilirubinemia and full term healthy newborn controls without hyperbilirubinemia on 2nd day after birth was collected and serum sample was separated. Total bilirubin and direct bilirubin were estimated by diazo method using Cobas6000 autoanalyzer. Indirect Bilirubin was obtained by calculation: Total bilirubin-conjugated bilirubin. Serum BDNF was estimated by sandwich immunoassay using commercially available ELISA kit [10].

Statistical analysis

All statistical analyses were conducted by using Statistical Package for the Social Sciences (SPSS V.17.0). The categorical variables were expressed in terms of percentages and Chi-square test was used for the analysis of these variables. Continuous variables were analysed by measures such as sample mean, standard deviation, and statistical significance was tested by student 't' test and Anova. Post Hoc (Bonferroni) was used to analyse the significance of difference of values between the groups. Correlation among the biochemical parameters was analysed by Karl Pearson's Correlation Analysis. Value of $p<0.05$ was considered as significance.

Results

The current study results are represented in Table 1 which shows Mean ± SD values of the gestational age, birth weight, total bilirubin, direct bilirubin, indirect bilirubin and BDNF in the 3 study groups. Table 2 depicts correlation of BDNF with total bilirubin in Group I, Group II and Group III. Figure 1 represents significant positive correlation between bilirubin and BDNF levels in preterm neonates. This observation suggests that with an increase in serum bilirubin levels there is a significant increase in serum BDNF levels ($p<0.05$) in preterm neonates (Group II)

Table 1: Study parameters in controls and patients

Parameters	Group I (n=30)	Group II (n=30)	Group III (n=30)	p
Gestational age in weeks	38.83 ± 0.79	34.57 ± 1.48*	39 ± 0.83***	<0.001
Birth weight in kg	2.89 ± 0.34	2.32 ± 0.30*	2.95 ± 0.42***	<0.001
Serum total bilirubin (mg/dl)	8.368 ± 4.51	11.80 ± 2.87*	13.19 ± 2.39**	<0.001
Serum direct bilirubin (mg/dl)	0.79 ± 0.44	0.78 ± 0.41	1.0 ± 0.58	0.144
Serum indirect bilirubin (mg/dl)	7.89 ± 4.44	10.99 ± 2.83*	12.19 ± 2.28**	<0.001
Serum BDNF (ng/ml)	155.18 ± 34.23	94.49 ± 50.89*	115.92 ± 54.08**	<0.001

Values were expressed in Mean ± SD, Group I: normal control, Group II: pre-term neonates, Group III: term neonate
**Significance of difference between Group I and Group II:p<0.05, **Significance of difference between group I and group III:p<0.05, ***Significance of difference between group II and group III:p<0.05*

Table 3: Correlation of BDNF with total bilirubin in Group I, Group II and Group III

Groups	r	p
Group I	0.123	0.517
GroupII	0.373	0.042*
Group III	0.208	0.269

**p<0.05-Significant, Group I: normal control, Group II: pre-term neonates, Group III: term neonate*

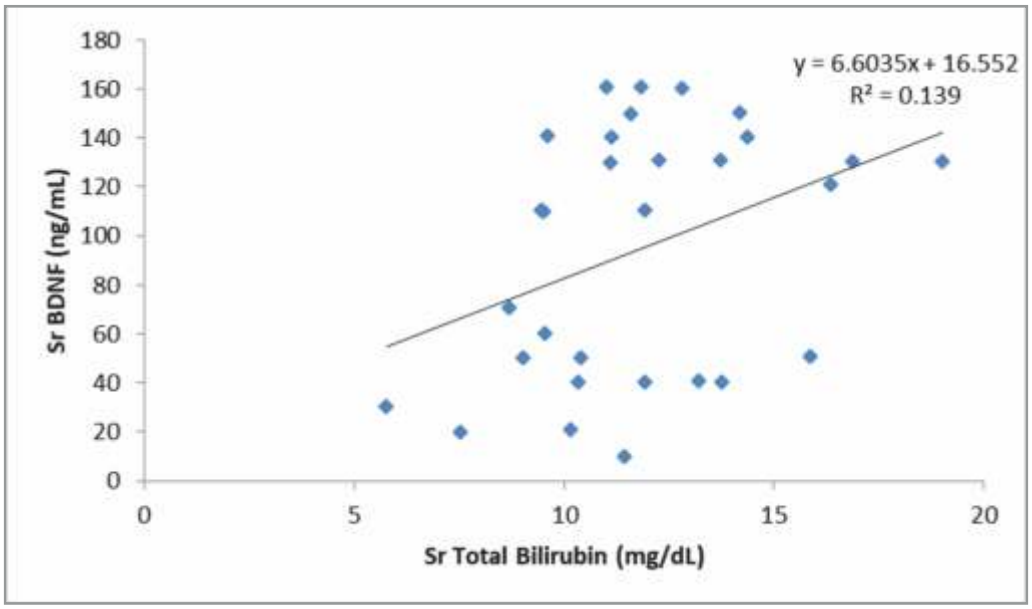

Figure 1: Correlation between bilirubin and BDNF levels in preterm neonates

Discussion

The present study observed that there was a significant decrease in serum levels of BDNF in pre-term and term neonates with hyperbilirubinemia when compared to controls (neonates without hyperbilirubinemia).

Birth weight of pre-term neonates was found to be the least when compared to term neonates with and without hyperbilirubinemia.The most common cause for a decrease in birth weight of preterm neonates could be preeclampsia in mothers [11] and also a deficiency in total calorie intake by the mothers. There was a reduction in the proportion of preterm neonates from 17.8% to 7.4%, an increase in birth weight by 496 g and an increase in gestational age by 4.3 days when mothers were given a 4 meal diet with adequate nutrients [12]. There was also a positive correlation of gestational age with serum BDNF levels in group I, a significant negative correlation of birth weight with serum BDNF levels in group III and a significant positive correlation of serum bilirubin with serum BDNF in group II.

Our study is also in concordance with reports published by Afify [5] and Malamitsi-Puchner *et al*., [9] who showed that there was an increase in BDNF levels with increasing gestational ages. This shows that BDNF is neuroprotective and has a role in developmental maturity of cortex, neuronal survival and synaptic plasticity that leads to improvement of synaptic connections [13].

BDNF is produced by the hippocampus and is an inhibitor of apoptosis of neurons. Its biologic function is synergistic to serotonin. It can cross the blood-brain barrier, so that the levels in the serum parallels the levels in the brain [14]. Therefore, the serum levels of BDNF gives a direct measure of the extent of brain damage.

In few patients, increased circulating bilirubin levels has resulted in ABE, CBE kernicterus, leading to permanent neurological injury to various subcortical brain structures [15]. At a

cellular level, unbound or 'free' bilirubin crosses the Blood Brain Barrier (BBB), where it activates glial cells, impairs myelination, and promotes neuronal apoptosis [16]. As reported earlier mature BDNF is critical to neuro protection in the neonatal brain, new-bornsmay be susceptible to brain injury on account of decreased brain BDNF levels [17].

Huppi *et al*. [18] established that human cortical gray matter increases by 4-fold between 29- and 35-weeks' gestation with increasing synaptic maturity. The increasing BDNF levels through this gestational age group may signify its role in human brain development. It is also shown that neurogenesis may be promoted in adult brain by infusion of BDNF inanimal experiments while a BDNF gene deletion showed early death after birth indicating the role of BDNF in postnatal survival [19]. Certain molecules, for instance, melatonin is reported to increase BDNF expression, possesses anti-apoptotic effects and simultaneously decreases the adverse effects of neonatal hyperbilirubinemia [20]. Therefore, it is evident that best clinical outcome can be achieved during the treatment of hyperbilirubinemia by increasing the BDNF expression.

Our reports are in agreement with earlier findings. Low levels of BDNF in preterm neonates may result in lower degrees of neuro protection in them. Preterm and term neonates with hyper-bilirubinemia showed significant decrease in BDNF compared to neonates without hyper-bilirubinemia which apparently indicates that bilirubin could have a role in decreasing BDNF levels. Interestingly there was a significant positive correlation between bilirubin and BDNF levels in preterm neonates- Preterm neonatal brain is vulnerable to free radical attack and plausible brain damage [21]. High levels of bilirubin within physiological limits in neonates, probably explains the role of bilirubin as an antioxidant at physiological levels.

Conclusion

Serum levels of BDNF could be a useful marker in neurological risk assessment in term and preterm neonates with hyperbilirubinemia which may aid in clinical decision making. A better understanding of the correlation between serum BDNF and bilirubin levels can be attained by continuing the study with neonates having kernicterus and neonates with hypoalbuminemia.

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