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Relation between serum neurotrophins at birth and development of bronchopulmonary dysplasia in premature infants

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Abstract

Neurotrophin concentrations were linked to preterm birth difficulties, birth weight and gestational age as a whole and/or to premature birth in the past. Neurotrophins in the blood of preterm infants and the development of bronchopulmonary dysplasia have been linked to long-term neurodevelopmental outcomes in this research. The research was done on 174 preterm neonates hospitalised to the neonatal intensive care unit (NICU) at Benha University Hospital between April 2019 and May 2020, after their parents signed an informed consent form. However, owing to kit limitations and for statistical correlation, a total of 90 cases were examined, 30 instances in each group being randomly assigned to a blinded group. The research was given the go light by the hospital's ethical scientific committee. Group 1 (preterm with BPD), Group 2 (preterm without BPD), and Group 3 (preterm without BPD) were each subdivided into 30 cases (Healthy preterm as a control group), Those who survived the first 24 hours of life were asked to come back for a follow-up visit to be enrolled in neurodevelopmental testing using Bayley Scales Of Infant and Toddler Development (BSID-III) at 24 months of age corrected age, and those who didn't were asked to come back for a second visit to be enrolled in the BSID-III. Results: Serum BDNF and NGF levels at birth in groups 1 and 2 were significantly higher than in groups 3 and 4, whereas levels in groups 3 were significantly lower than in groups 1 and 2. Invasive mechanical ventilation and supplementary oxygenation, as well as the development of bronchiolitis obliterans (BPD), are associated with lower BDNF serum concentrations at birth. Neurodevelopmental outcomes may be predicted by the level of NGF in a baby's blood at birth. We can increase our capacity to predict at birth whether a baby will be diagnosed with BPD and their long-term neurodevelopmental outcomes by measuring the concentration of serum neurotrophic factors in preterm neonates.

Keywords: neurotrophins, development, bronchopulmonary dysplasia, BPD, premature.

1. Introduction

Many conditions and diseases can occur in premature infants, including brain injury (e.g. intraventricular haemorrhage (IVH), periventricular leukomalacia (PVL), retinopathy of prematurity (ROP), bronchopulmonary dysplasia, neonatal respiratory distress syndrome, necrotizing enterocolitis (NEC), and anaemia [1].

Prematurity-related problems, birth weight, gestational age, and preterm delivery in general have all been linked to neurotrophin concentrations [2], [3].

NGF, BDNF, NT-3, and NT-4 are all members of the traditional neurotrophin family, which consists of four molecules that are structurally and functionally linked (NT-4) [4].

The neurotrophin family includes brain-derived neurotrophic factor (BDNF), which is involved in central and peripheral neuron maintenance, survival, and differentiation. Immune cells, epithelial cells, and smooth muscle cells all benefit from BDNF [5–8] by surviving and being activated in the lung.

NGF, the first neurotrophin to be discovered, has long had a prominent place in developmental biology because of its role in preventing programmed cell death throughout embryonic and postnatal development [9].

Sympathetic and sensory target organs produce and release NGF, which is subsequently taken up in nerve terminals by receptor-mediated endocytosis and delivered to neuronal cell bodies through axons [10], [11].

Currently, neonatologists are unable to accurately estimate the prognosis of preterm newborns, establish specific expectations for the neonatal period, or explore early adoption of more aggressive therapy [12].

At Benha University Hospital's newborn intensive care unit, this research attempted to identify the link between the development of bronchopulmonary dysplasia and long-term neurodevelopmental outcomes in preterm neonates with high levels of serum neurotrophins at delivery.

2. Patients and methods

This prospective follow up study was conducted on 174 preterm neonates admitted in neonatal intensive care unit (NICU) at Benha University Hospital in the period from April 2019 to May 2020, after obtaining informed consents from their parents before enrollment in the study. But 90 cases were studied, 30 cases in each group in a blind selection due to kits limitation and for statistical correlation. The study was approved by ethical scientific committee of Benha University hospital.. The study was approved by ethical scientific committee of Benha University hospital.

They were divided in a retrograde manner into 3 groups (each group included 30 case):

Group 1(preterm with BPD): it included 30 cases

Preterm requiring a fraction of inspired oxygen >0.21 for at least 28 days was diagnosed with BPD according to criteria of [13].

Group 2 (preterm without BPD): - included 30 cases not fulfilling criteria of [13].

Group 3(Healthy preterm): -

This group included 30 preterm healthy neonates not needing oxygen therapy admitted due to low birth weight as a grower to be as a control group.

Exclusion criteria:

- Preterm with suspected Hypoxic Ischemic Encephalopathy (HIE).
- Preterm who had underwent transfusion at any time prior to sample collection.
- Neonates presenting with congenital anomalies, renal malformations, chromosomal abnormalities and congenital infection.

All participants were subjected to full history taking, complete clinical examination and laboratory investigations including serum neurotrophins: including Nerve Growth Factor (NGF), Brain Derived Growth Factor (BDNF) within 24 hours of age, Peripheral blood samples were collected using sterile technique per routine NICU standard of care. All samples were drawn within the first 24 hours after birth. A volume of 3 mL of blood were collected and sample is allowed to clot for serum coagulation at room temperature 10-20 min then centrifugation 20-min at the speed of 2000-3000 r.p.m. The supernatant was collected and placed into sterile Eppendorf tubes and stored at -80 C until being measured by ELISA kits supplied by Sun red biotechnology company in Bao Shan Qu, Shang Hai Shi, China.

Survived cases from each group were asked to attend for follow up visit to be enrolled in neurodevelopmental testing using Bayley Scales Of Infant and Toddler Development (BSID-III) at 24 months of age corrected age.[14], [15]**.**

The Bayley-III is commonly used to identify developmental delay in clinical groups and high-risk children by comparing the abilities of the child to a normative age-matched sample of children.

The Bayley Scales of Infant and Toddler Development is a gold standard tool that assesses cognitive, language, motor, social-emotional development and adaptive behavior in children aged 1–42 months.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**. (**Armonk, NY: IBM Corp**)** Qualitative data were described using number and percent. The Shapiro-Wilk test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level. The used tests were; Chi-square test for categorical variables, to compare between different groups. Fisher's Exact or Monte Carlo correction; Correction for chi-square when more than 20% of the cells have expected count less than 5. F-test (ANOVA); For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons. Kruskal Wallis test; For not normally distributed quantitative variables, to compare between more than two studied groups, and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons. Student t-test; For normally distributed quantitative variables, to compare between two studied groups. Pearson coefficient; To correlate between two normally distributed quantitative variables. Receiver operating characteristic curve (ROC); It is generated by plotting sensitivity (TP) on Y axis versus 1-specificity (FP) on X axis at different cut off values. The area under the ROC curve denotes the diagnostic performance of the test. Area more than 50% gives acceptable performance and area about 100% is the best performance for the test. The ROC curve allows also a comparison of performance between two tests.

3. Results

This prospective follow up study was conducted on 90 preterm neonates. They were divided in a retrograde manner into 3 groups (each group included 30 case): Group 1(preterm with BPD), group 2 (preterm without BPD) and group 3 (Healthy preterm as a control group). Regarding gestational age and birth weight there was statistically significant decrease in group1 than group 2,3 and significant decrease in group 2 in comparison with group 3. Regarding parity there was statistically significant increase in multiple births among group 1 & 2 compared to group 3. There was no significant statistical difference between the three groups in other demographic data as gender, mode of delivery and maternal age. table 1

As regard serum BDNF at birth, there was statistically significant decrease in group 1 than 2 and in group 2 than in group 3. As regard serum NGF at birth, there was statistically significant decrease in group 1 than 2 and in group 2 than in group 3, table 2

Table 3 shows correlation between serum BDNF at birth and NGF at birth in the three studied groups and different parameters. There was significant negative correlation between serum BDNF at birth and gestational age in the three studied groups. There was significant negative correlation between serum BDNF at birth and invasive ventilation days in group 1 and group 2. There was significant negative correlation between serum BDNF at birth and supplemental oxygen days in group 1 and group 2.

Table 4 Correlation between serum BDNF at birth and serum NGF at birth in the three studied groups and cognitive composite score, language composite score and Motor composite score done at 24-month-old according to Bayley scale of infant development 3rd edition and total 30 cases attended 10 from each group and there was significant statistical positive correlation between serum NGF at birth and cognitive composite score, language composite score only in group 1 but no significant statistical correlation between serum NGF at birth and cognitive composite score, language composite score in group 2 and 3. There was no significant statistical correlation between serum NGF at birth and motor composite score in the three studied groups. There was no significant statistical correlation between serum BDNF at birth and cognitive composite score, language composite score and motor composite score in the three studied groups.

Table (1) Comparison between the three studied groups according to demographic data

	Group 1 (n = 30) No.	%	Group 2 (n = 30) No.	%	Group 3 (n = 30) No.	%	Test of sig.	p
Gender								
Male	12	40.0	14	46.7	19	63.3	χ^2= 3.467	0.177
Female	18	60.0	16	53.3	11	36.7		
Gestational age (weeks								
Min. – Max.	29.0 – 31.0		29.0 – 31.0		33.0 – 35.0		F= 323.70	<0.001*
Mean ± SD.	29.40 ± 0.56		29.87 ± 0.73		33.90 ± 0.92			
Median (IQR)	29.0 (29.0 – 30.0)		30.0 (29.0 – 30.0)		34.0 (33.0 – 35.0)			
Sig. bet. grps.	$p_1 = 0.048^*$, $p_2<0.001^*$, $p_3<0.001^*$							
Birth weight (grams)								
Min. – Max.	1100.0 – 1800.0		1260.0 – 1820.0		1750.0 – 2605.0		F= 191.037*	<0.001*
Mean ± SD.	1265.50 ± 136.24		1473.67 ± 134.30		2160.83 ± 258.50			
Median (IQR)	1232.5 (1180.0 – 133.0)		1465.0 (1370.0 – 1550.0)		2100.0 (1950.0 – 2420.			
Sig. bet. grps.	$p_1<0.001^*$, $p_2<0.001^*$, $p_3<0.001^*$							
Parity								
Single	19	63.3	18	60.0	30	100.0	χ^2= 15.535*	<0.001*
Multiple births	11	36.7	12	40.0	0	0.0		
Sig. bet. grps.	$p_1 = 0.791$, $p_2=1.000$, $p_3<0.001^*$							
Delivery method								
Vaginal delivery	7	23.3	15	50.0	9	30.0	χ^2= 5.118	0.077
Cesarean section	23	76.7	15	50.0	21	70.0		
Maternal age (years)								
Min. – Max.	23.0 – 29.0		22.0 – 30.0		24.0 – 29.0		F= 0.662	0.518
Mean ± SD.	26.23 ± 1.96		26.80 ± 2.16		26.43 ± 1.65			
Median (IQR)	26.0 (25.0 – 28.0)		27.0 (26.0 – 29.0)		26.0 (25.0 – 28.0)			

Table (2) Comparison between the three studied groups according to serum BDNF at birth and serum NGF at birth.

	Group (n = 30)	Group (n = 30)	Group (n = 30)	F	p
Serum BDNF (pg/ml) at birth					
Min. – Max.	2800.0 – 8632.0	4547.0 – 16548.0	9254.0 – 23861.0	47.408*	<0.001*
Mean ± SD.	5557.7 ± 1772.5	10105.7 ± 4491.0	13405.9 ± 2483.6		
Median (IQR)	5655.0 (3800.0 – 7080.0)	9336.5 (5876.0 – 14599.0)	12878.0 (12454.0 –14280.0)		
Sig. bet. grps.	$p_1<0.001^*$, $p_2<0.001^*$, $p_3<0.001^*$				
Serum NGF (pg/ml) at birth					
Min. – Max.	423.0 – 3540.0	1765.0 – 10347.0	1079.0 – 10993.0	39.651*	<0.001*
Mean ± SD.	1394.3 ± 749.6	5336.2 ± 2647.6	6982.3 ± 3338.3		
Median (IQR)	1155.0 (942.0 – 1756.0)	4797.0 (3215.0 – 6754.0)	5438.0 (4621.0 – 10354.0)		
Sig. bet. grps.	$p_1<0.001^*$, $p_2<0.001^*$, $p_3 = 0.033^*$				

Table (3) Correlation between serum BDNF at birth and serum NGF at birth in the three studied groups and different parameters.

		Serum BDNF at birth			Serum NGF at birth		
		Group 1 (n = 30)	Group 2 (n = 30)	Group 3 (n = 30)	Group 1 (n = 30)	Group 2 (n = 30)	Group 3 (n = 30)
Gestational age (weeks)	**r**	-0.482	-0.525	-0.397	0.237	-0.158	0.197
	P	0.007*	0.003*	0.030*	0.207	0.404	0.296
Clinical presentation and Downe score	**r**	0.245	0.041	–	-0.209	-0.077	–
	P	0.192	0.830	–	0.267	0.686	–
Grades of RDS in CXR	**r**	-0.237	-0.284	–	-0.032	-0.114	–
	P	0.207	0.129	–	0.866	0.550	–
Invasive ventilation (days)	**r**	-0.445	-0.382	–	-0.013	0.056	–

Days on CPAP	**P**	0.014*	0.037*	–	0.946	0.769	–
	r	-0.022	-0.178	–	0.116	-0.105	–
Supplemental oxygen (days)	**P**	0.907	0.346	–	0.540	0.580	–
	r	-0.430	-0.406	–	0.126	-0.329	–
Length of hospital stay (days)	**P**	0.018*	0.026*	–	0.508	0.076	–
	r	0.220	-0.343	-0.330	-0.074	-0.199	0.000
	P	0.242	0.063	0.075	0.699	0.292	0.998

Table (4) Correlation between serum BDNF at birth and serum NGF at birth in the three studied groups and cognitive composite score, language composite score and Motor composite score done at 24-month-old according to Bayley scale of infant development 3rd edition.

		Serum BDNF at birth			**Serum NGF at birth**		
		Group 1 (n = 10)	**Group 2 (n = 10)**	**Group 3 (n = 10)**	**Group 1 (n = 10)**	**Group 2 (n = 10)**	**Group 3 (n = 10)**
Cognitive composite score (CCS)	**r**	-0.356	-0.206	0.291	0.735	0.375	0.007
	P	0.313	0.569	0.414	0.015*	0.285	0.985
Language composite score (LCS)	**r**	0.347	-0.523	0.553	0.661	0.095	0.043
	P	0.327	0.121	0.097	0.037*	0.795	0.906
Motor composite score (MCS)	**r**	0.440	-0.178	0.434	0.025	0.057	-0.187
	P	0.203	0.624	0.210	0.944	0.876	0.604

IQR: **Inter quartile range** SD: **Standard deviation**
F: **F for ANOVA test**, pairwise comparison between each 2 groups was done using **Post Hoc Test (Tukey)**
p: p value for comparing between the studied groups
p_1: p value for comparing between **Group 1** and **Group 2**
p_2: p value for comparing between **Group 1** and **Group 3**
p_3: p value for comparing between **Group 2** and **Group 3**
Group 1: Preterm with BPD **Group 2: Preterm without BPD** **Group 3: Healthy preterm**
r: Pearson coefficient *: Statistically significant at $p \leq 0.05$

ROC curve was performed to assess the performance of serum BDNF at birth and serum NGF at birth to discriminate preterm group with BPD (n = 30) from preterm group without BPD (n = 30); As regard serum BDNF at birth there was a statistically significant P value (<0.001) with AUC was (0.781) and Confidence Interval (0.666 – 0.896) and cut off point was (≤6690 pg/ml) with sensitivity (73.33) and specificity (66.67) and PPV was (68.7) and NPV was (71.4). As regard serum NGF at birth there was a statistically significant P value (<0.001) with AUC was (0.963) and Confidence Interval (0.925 – 1.0) and cut off point was (≤2356 pg/ml) with sensitivity (93.33) and specificity (83.33) and PPV was (84.8) and NPV was (92.6). figure 1

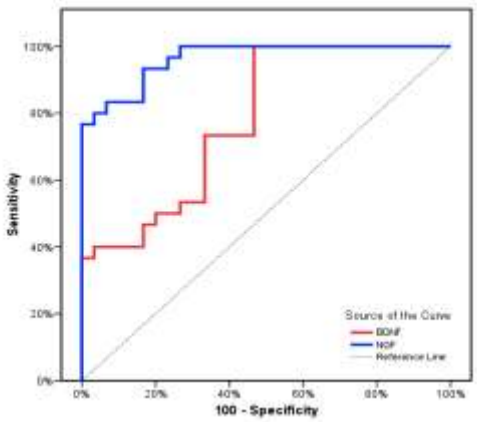

Fig.(1) ROC curve for serum BDNF at birth and serum NGF at birth to discriminate group 1 (n = 30) from group 2 (n = 30)

ROC curve was performed to assess the performance of serum BDNF at birth and serum NGF at birth to discriminate group 1 from group 3; As regard serum BDNF at birth there was a statistically significant P value (<0.001) with AUC was (1.000) and Confidence Interval (1.0 – 1.0) and cut off point was (≤8632 pg/ml) with sensitivity (100.0) and specificity (100.0) and PPV was (100.0) and NPV was (100.0). As regard serum NGF at birth there was a statistically significant P value (<0.001) with AUC was (0.964) and Confidence Interval (0.919 – 1.0) and cut off point was (≤3098 pg/ml) with sensitivity (93.33) and specificity (86.67) and PPV was (87.5) and NPV was (92.9). figure 2

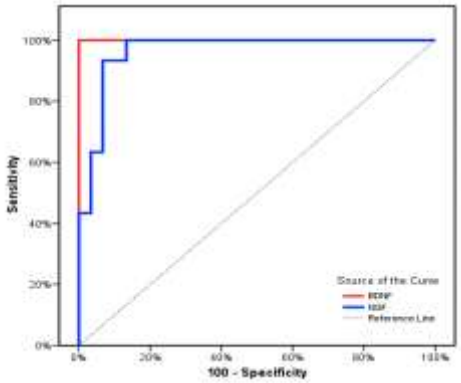

Fig.(2) ROC curve for serum BDNF at birth and serum NGF at birth to discriminate group 1 (n = 30) from group 3 (n = 30)

4. Discussion

When it comes to serum BDNF levels at delivery, preterm with BPD had significantly lower levels than preterm without BPD (10105.7 pg/ml) or control group (13405.9 pg/ml), both of which had p0.001 statistical significance.

Serum BDNF concentration at birth differs significantly across the three groups, with preterm infants with BPD having significantly lower serum BDNF than preterm infants without BPD and term infants (P 0.001).

Preterm neonates had lower levels of BDNF than term newborns (SMD = -0.32; 95 percent CI: -0.59, -0.06; p = 0.014) in a comprehensive review and meta-analysis "Neuroprotection Factor Levels in Preterm Infants" [16].

An experimental group of babies with BPD was shown to have a reduced BDNF serum concentration, which is consistent with prior findings that the high-affinity BDNF receptor (TrkB) is required for proper lung development. There are anatomical abnormalities in the lungs that are comparable to those seen in BPD when this gene is selectively mutated, as well as altered airway innervation and breathing control impairments.

Preterm children in the BPD group had significantly lower levels of serum NGF at birth (1394.3 749.6 pg/ml) than preterm children in the control group (5336.2 2647.6 pg/ml) and the group without BPD (6982. 3 3338.3 pg/ml), p0.001.

Preterm babies with BPD had considerably lower serum NGF than preterm infants without BPD or term newborns, as reported by [12], who also found significant differences in NGF serum concentration between groups at birth (P = 0.010).

Full-term newborns had greater urine NGF levels than preterm and IUGR neonates, according to Aisa et al. [18]. Such changes were statistically significant when compared to all IUGR babies or the preterm ones of the impaired neurodevelopment subgroup. In addition, a comparison of the two groups' preterm and all-IUGR categories shows a substantial decrease in people with inferior neurodevelopmental outcomes.

Twins and triplets had lower predicted GA (mean 3.0 weeks; 95% confidence interval 1.6-4.3 weeks) at a given BDNF concentration than singleton newborns, which is in line with the findings of [12], who found a moderately strong positive association between serum BDNF concentration and GA (Spearman Correlation Coefficient = 0.51). They also found that high levels of serum BDNF were related with a reduced risk of any ventilator or oxygen usage (P = 0.009 and P 0.015, respectively). There was no correlation between race, ethnicity, or maternal smoking and BDNF concentrations at birth (P = 0.27, 0.80, and 0.19, respectively). Male newborns had lower BNDF than female infants in univariable analysis (P = 0.001), and this difference persisted even after controlling for multiple birth and GA (P = 0.003).

A prior study of cord blood samples showed that the serum BDNF levels are greatest in children born at or before 36 weeks gestational age (GA) and are lowest in those born at or after 24 to 28 weeks (GA). Our findings support that finding [19].

There were no significant associations between the gestational week, birth weight, and clinical comorbidity of newborns with BDNF effect sizes in a systematic review and meta-analysis by Krey et al. [16].

Rao et al. [2] found that BDNF concentrations were positively linked with the length of membrane rupture (r = 0.43, p 0.04) in newborns 32 weeks gestational age. Complete prenatal steroids treatment increased BDNF levels in babies by 1461 (553–2064) and 281 (171–536) pg/mL, respectively, in comparison to partial prenatal steroids treatment.

This research found a statistically significant positive link between serum NGF at birth and cognitive composite score (CCS) and linguistic composite score (LCS) in the preterm with BPD group, but there was no statistical correlation between serum NGF at birth and CCS and LCS in other groups. In each of the three groups, there was no statistically significant link between the level of NGF in a baby's blood at birth and the motor composite score (MCS).

As Simpson et al. [12] found, serum NGF levels tested at birth were strongly associated with LCS (P = 0.001), while there was no significant link with CCS (P = 0.010) or MCS (P = 0.60).

It has been shown that urine NGF and brain volume are substantially associated in preterm and IUGR patients with poor neuro-development, according to Aisa et al. [18]. There was a substantial difference in urine NGF levels between the subgroups with various outcomes, thus

an AUC was performed to see whether urinary NGF may be used to predict whether or not a neonate is at risk of neurodevelopmental impairment. Accuracy in diagnosis was good, with an AUC of 0.925 (CI 0.854-0.1002) and p-value of 0.04.

5. Conclusion

Normal respiratory and nervous system development and function depend on the physiological expression of neurotrophins such nerve growth factor (NGF) and brain-derived growth factor (BDNF), as well as their high-affinity tropomyosin receptor kinase A and B (TrkA and TrkB) receptors. Premature infants that have a high concentration of neurotrophic factors in their blood are more likely to be diagnosed with BPD and have worse clinical outcomes. Invasive mechanical ventilation and supplementary oxygenation are associated with a higher levels of BDNF in the blood at delivery. Neurodevelopmental outcomes may be predicted by the level of NGF in a baby's blood at birth.

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