Brain-Derived Neurotrophic Factor (BDNF), and Neurotrophin 3 (NT3) Levels in Newborn Cord Sera

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Abstract:
During embryonal development, neuronal death occurs only by apoptosis and not by necrosis. Apoptotic neuronal loss may be responsible for altered brain development associated with prematurity and perinatal insults. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF), and neurotrophin 3 (NT3) play crucial roles in protecting neurons from entering or progressing along an apoptotic pathway. The aim of this work was to measure BDNF and NT3 levels at different gestational ages in human umbilical cord blood. In addition, we searched for differences in BDNF and NT3 levels in the presence or absence of factors that may affect intrauterine conditions and thus neurodevelopmental outcome. We collected 80 samples of cord blood and categorized them accordingly into three gestational age groups: group 1 (24-30 weeks), group 2 (31-36 weeks), and group 3 (37-42 weeks). BDNF and NT3 levels were determined by ELISA. The BDNF levels were 798.3±492.5, 1401±650.8, and 2236.6±376.8 pg/ml in group 1, group 2, and group 3, respectively, with a significant difference between the 3 groups (p=0.0001). In contrast, NT3 levels did not show significant change across gestational ages (p=0.2). NT3 levels also did not correlate with BDNF levels across gestational ages (r=0.23; p=0.27). The presence of premature rupture of membranes, chorioamnionitis, pregnancy-induced hypertension, or small for gestational age did not alter either BDNF or NT3 levels significantly. BDNF and NT3 levels were significantly higher in samples from subjects whose mothers received two doses of antenatal steroids compared with those who received only one dose of steroids, and those with no antenatal steroids (p=0.001, and p=0.04).

Conclusion: Cord blood levels of BDNF may reflect the degree of neural maturity in premature infants. Increased BDNF and NT3 levels may also mediate improved neurodevelopmental outcome in infants who received antenatal steroids.

Abbreviations:

Introduction:
As a result of advances in perinatal management, survival for infants born before 32 weeks of gestation is continuing to increase. However, rates of neurodevelopmental impairments have remained substantially unchanged,1 and the long-term outcome of extremely immature infants remains a major concern. For example, school performance of infants weighing <1000 g is suboptimal when compared with both their term peers and with infants weighing between 1000 and 1500 g.2,3 Most neurodevelopmental impairments are likely to be the consequence of brain damage of perinatal origin. Multiple factors play a role in neurodevelopmental outcome of these prematurely born infants. Chorioamnionitis, PIH, and PROM significantly alter intrauterine conditions that lead to SGA status, and also may alter the neurodevelopmental outcome of prematurely born infants.4-6 Mothers expected to have premature delivery are often given antenatal steroids to improve pulmonary maturity. These steroids have been shown to improve neurodevelopmental outcome.7 Infants born before 32 weeks gestation have less cortical gray matter when measured at corrected term postconceptional age compared with full term infants measured at birth.8 The mechanism of the altered development is predicted to be a process of apoptotic neuronal loss or damage. The actual volume of cortical gray matter of infants born between 29 and 35 weeks gestation shows a progressive increase with length of gestation up to 4-fold.9 This period is a critical phase of neurodevelopment and may be an important consideration for infants born prematurely. It is thus important to analyze the neurophysiologic milieu at this stage of development. Neurotrophic factors play crucial roles in...
neuroprotection. Neurotrophins promote survival and can reduce apoptosis in many populations of neurons. Nerve growth factor, BDNF, and NT3 are neurotrophins that act on tyrosine kinase (Trk) A, TrkB and TrkC receptors, respectively. In addition to antiapoptotic activities, neurotrophins play important roles in axon growth during development, higher neuronal functions, morphologic differentiation, and neurotransmitter expression. Thus, neurotrophins may play important roles in antenatal and postnatal brain development. However, data regarding the presence and influence of neurotrophins in prematurely born infants are insufficient. The cerebrospinal fluid levels of BDNF were higher when measured in infants who had hypoxic-ischemic insults compared with their normal counterparts. In addition, BDNF has been demonstrated to decrease tissue loss in brain when administered after hypoxic-ischemic injury in neonatal animals. Circulating BDNF levels correlate with cortical BDNF levels in newborn rats. Nerve growth factor levels measured in umbilical cord blood were higher in samples from infants born at term compared with those obtained from preterm infants. Because the blood-brain barrier at this stage is still immature, these blood levels may also represent neurotrophin concentrations in the central nervous system. The aim of this work was to measure BDNF and NT3 levels at different gestational ages in human umbilical cord blood to investigate the hypothesis that neurotrophin levels differ at different gestational ages. In addition, we looked at differences in BDNF and NT3 levels in the presence or absence of factors that may affect intrauterine conditions and thus neurodevelopmental outcome.

Subjects and Methods:

This study was carried out at the Pediatric Department of Al-Minya University Hospital in the period from July to October 2004. The study included 80 newborns, selected from Delivery Room, Gynecology and Obstetric Department of Al-Minya University Hospital which cares for approximately 4200 deliveries per year, and the parents of the subjects examined gave informed consent. Clinical data, including birth weight, sex, head circumference, and gestational age at birth were recorded. Antenatal history regarding PROM, chorioamnionitis, PIH, SGA status, and antenatal steroids was obtained. PROM was diagnosed when a clinically apparent leakage of amniotic fluid was confirmed in the patients who arrived thinking fluid was leaking without a preceding uterine contraction. Clinical chorioamnionitis was defined according to the criteria proposed by Gibbs et al. The diagnosis required a temperature elevation to 37.8°C and two or more of the following criteria: uterine tenderness, malodorous vaginal discharge, maternal tachycardia, fetal tachycardia, and leukocytosis (white blood cell count >15,000/mm³). Gestational hypertension was defined as diastolic pressure ≥90 mm Hg on two occasions at least four hours apart or a single reading of ≥110 mm Hg from 20 weeks' gestation onwards in a previously normotensive woman. Pre-eclampsia was defined as gestational hypertension plus proteinuria of ≥0.3 g/24 hours. The SGA status was defined as birth weights <10 centiles. Gestational age was estimated at birth by the duration of amenorrhea combined with early ultrasound findings, and after delivery by modified Ballard Score of combined physical and neurological examination. Based on gestational age assessment, studied newborns were classified into the following 3 groups: Group 1: 24 preterm newborns “24-30 weeks gestational age.” Group 2: 34 preterm newborns “31-36 weeks gestational age.” Group 3: 22 full term newborns “37- 42 weeks gestational age.” In our hospital, obstetricians initiate antenatal corticosteroid therapy (intramuscular dexamethasone) to enhance lung maturation of the fetus in women between 24 and 34 weeks of gestation in cases of threatened preterm labor, antepartum hemorrhage, PROM, or any condition requiring elective preterm delivery. We found that 48 of 80 studied newborns had a gestational age between 24 and 34 weeks, mothers of 15 of them received two doses of antenatal steroids(one week apart), and mothers of another 15 of them received one dose 24 hours before delivery, while mothers of 18 newborns did not receive antenatal steroids. The dose of I.M dexamethasone was agreed to be 6-8 mg/12 hours x 2 consecutive doses. This may only be repeated (as a rescue dose) if imminent delivery is expected more than 7 days following the last injection. Umbilical cord blood samples were collected from the studied newborns just after delivery. The sample collection was done using complete aseptic precautions. A wide bore needle was inserted into an umbilical vessel, and approximately 5 ml of blood was drawn into a sterile syringe. This blood then was transported to the blood bank, where it was centrifuged to separate the serum. The samples were stored at -80°C. BDNF and NT3 levels were determined by using ELISA kits from R & D Systems in triplicate. Appropriate controls were used to eliminate errors caused by background. One control was without biotinylated secondary antibody. This control accounts for the endogenous peroxidase...
activity of plasma and Hb (in hemolyzed samples). A second control was without the antigen and accounts for the possibility of contamination of either primary or secondary antibody with BDNF.

**Statistical Methods:**

After collection of data, they were added and entered into a personal computer. Analysis of the data was done using SPSS (Statistical Package for the Social Sciences). The following statistical tests were used:
1. Mean and standard deviation (SD) to describe quantitative data.
2. Student t test was used to compare between two groups as regards parametric data.
3. ANOVA was used for comparison between more than two groups.
4. Pearson correlation was used to correlate two quantitative variables.

For all tests, a probability (p) of less than 0.05 was considered significant.

**Results:**

**Subject description:**
The differences among groups 1, 2, and 3 were statistically significant for birth weight and head circumference for the subject population (table I). Distribution of males and females was not significantly different among the gestational age groups. The differences among the occurrences of PROM, chorioamnionitis, PIH, or SGA were not statistically significant in different gestational age groups (table I).

**Neurotrophin levels at different gestational ages:**
The differences between BDNF levels when compared across gestational age groups demonstrated a significant increase with increased gestational age ($p=0.0001$)(table II). The BDNF level differences between groups 1 and 2 ($p=0.0002$), groups 2 and 3 ($p=0.0001$), and groups 1 and 3 ($p=0.0001$) were also statistically significant. In contrast, NT3 levels in group 1, group 2, and group 3 did not differ across gestational age groups ($p=0.2$) (table II). BDNF levels seem to increase with increasing gestational ages, whereas NT3 levels remain unchanged. NT3 levels also did not correlate with BDNF levels across gestational ages ($r=0.23$; $p=0.27$).

**Neurotrophin levels and clinical variables:**

We compared BDNF and NT3 levels in the presence or absence of clinical variables. BDNF and NT3 levels were higher in females than in males, but did not reach statistical significance ($p=0.1$, and $p=0.17$) (table III). Similar trends, without statistical significance, were observed when neurotrophin levels of females and males were compared within gestational groups. The presence of PROM, chorioamnionitis, PIH, or SGA did not seem to alter either BDNF or NT3 levels significantly (table III).

**Neurotrophin levels and antenatal steroids:**

BDNF and NT3 levels were significantly higher ($p=0.001$, and $p=0.04$) in samples from subjects whose mothers received two doses of antenatal steroids compared with those who received only one dose of steroids, and those with no antenatal steroids(table IV).

![Table I: Subject description (n=80)](data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAAfAAAAAASAAMAAAAMzKuAAAAGXRFWHRTb2Z0d2FyZQBBZG9iZSBJbWFnZVJlYWR5ccllPAAAAyFpVFh0WE1MOmNvbS5hZG9iZS54bXAAAAAAADw/eHBhY2tldCBiZWdpbj0i77u/IiBpZD0iVzVNME1wQ2VoaUh6cmVTek5UY3prYzlkIj8+IDx4OnhtcG1ldGEgeG1sbnM6eDw8P306TeXebTgAAAA持久AAAA

![Table II: Serum levels of BDNF and NT3 in studied groups](data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAAfAAAAAASAAMAAAAMzKuAAAAGXRFWHRTb2Z0d2FyZQBBZG9iZSBJbWFnZVJlYWR5ccllPAAAAyFpVFh0WE1MOmNvbS5hZG9iZS54bXAAAAAAADw/eHBhY2tldCBiZWdpbj0i77u/IiBpZD0iVzVNME1wQ2VoaUh6cmVTek5UY3prYzlkIj8+IDx4OnhtcG1ldGEgeG1sbnM6eDw8P306TeXebTgAAAA持久AAAA
Table III: Serum levels of BDNF and NT3 regarding clinical variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>BDNF (pg/ml)</th>
<th>NT3 (pg/ml)</th>
<th>p value</th>
<th>BDNF (pg/ml)</th>
<th>NT3 (pg/ml)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: Male (n=35)</td>
<td>1290±539.7</td>
<td>249.7±114.2</td>
<td>0.1</td>
<td>311.5±52.8</td>
<td>325.6±54.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Female (n=45)</td>
<td>1450±698.6</td>
<td>269.4±109.5</td>
<td>0.17</td>
<td>325.6±54.5</td>
<td>318.5±55</td>
<td>0.14</td>
</tr>
<tr>
<td>PROM: Present (n=20)</td>
<td>998.6±515</td>
<td>299.3±49.8</td>
<td>0.12</td>
<td>326±59.8</td>
<td>349.8±60.8</td>
<td>0.07</td>
</tr>
<tr>
<td>Absent (n=60)</td>
<td>1203±477.3</td>
<td>334.6±66.5</td>
<td>0.12</td>
<td>355.2±69.7</td>
<td>368.5±75</td>
<td>0.12</td>
</tr>
<tr>
<td>Chorioamnionitis: Present (n=11)</td>
<td>1178±548.2</td>
<td>334.6±66.5</td>
<td>0.06</td>
<td>326±59.8</td>
<td>349.8±60.8</td>
<td>0.07</td>
</tr>
<tr>
<td>Absent (n=69)</td>
<td>1384±514.3</td>
<td>355.2±69.7</td>
<td>0.12</td>
<td>368.5±75</td>
<td>368.5±75</td>
<td>0.12</td>
</tr>
<tr>
<td>PIH: Present (n=20)</td>
<td>1818.7±647.5</td>
<td>326±59.8</td>
<td>0.12</td>
<td>349.8±60.8</td>
<td>368.5±75</td>
<td>0.12</td>
</tr>
<tr>
<td>Absent (n=62)</td>
<td>1999±564.7</td>
<td>349.8±60.8</td>
<td>0.07</td>
<td>368.5±75</td>
<td>368.5±75</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table IV: Serum levels of BDNF and NT3 regarding antenatal steroid administration to mothers of newborns with gestational age between 24 and 34 weeks

<table>
<thead>
<tr>
<th>Variable</th>
<th>Antenatal steroids</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 doses (n=15)</td>
<td>1 dose (n=15)</td>
</tr>
<tr>
<td>BDNF (pg/ml): Mean±SD</td>
<td>1498±580.8</td>
<td>1053.7±515.5</td>
</tr>
<tr>
<td>NT3 (pg/ml): Mean±SD</td>
<td>295.5±130.4</td>
<td>249.2±44</td>
</tr>
</tbody>
</table>

*p<0.05 is significant.

Discussion:

Neurotrophins are one group of factors responsible for neuroprotection. Multiple studies have demonstrated various roles for neurotrophins in the prevention of neuronal apoptosis and various neuronal functions in animal models and adult humans. In addition, neurotrophin levels have been quantified in infants born with hypoxic-ischemic encephalopathy, and in those with mental retardation and autism. These studies demonstrated a rise in BDNF levels associated with both clinical situations.

In the present study BDNF levels increase with increasing gestational ages. A similar finding was also observed by Malamitsi-Puchner et al. Although BDNF and NT3 knockout animal models have demonstrated deficiencies mainly of the peripheral nervous system, their role in central nervous system development is being elucidated using double knockout and over expression experiments. BDNF has been shown to be responsible for developmental maturity of cortex and synaptic plasticity leading to refinement of connections. Increased expression of BDNF within the cortex has also been correlated to decreases in reelin expression. Reelin is an extracellular matrix molecule important in early cortical organization and development of the "inside-out" layering pattern. Decreased levels of reelin coincide with developmental maturity and the elicitation of synaptogenesis. Because Huppi et al. 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 the role played by BDNF during this phase of human brain development.

NT3 is also an important neurotrophin for a developing brain. NT3 and BDNF have similar functions related to neuronal survival. In fact, they tend to complement each other in their actions. However, some of the functions are distinct to each molecule. Despite all of these important functions, NT3 levels seem to remain unaltered, in our data, across the gestational age groups studied. This may be explained by the fact that neuronal responsiveness to the different neurotrophins changes with developmental changes, and thus, compensatory changes in neurotrophin expression may be required for proper balance of their actions. NT3 is important for multiplication of neuronal progenitors.

Thus, NT3 levels may have been higher in an earlier gestational period, when progenitor multiplication is at its peak.

Prematurely born infants had better survival and neurologic outcome when antenatal steroids were given. Higher levels of both neurotrophins were observed when mothers were given antenatal steroids. The results of Nitin et al. were also in agreement with ours. One possibility is that the improved developmental outcome may be mediated through increased availability of neurotrophins to these premature brains. Alternatively, it is also possible that improved neuronal maturity after antenatal steroids may induce increased neurotrophin secretion and further improvement in...
neurodevelopmental outcome. Thus, it may be interesting to study the presence and significance of these neurotrophins in premature infants postnatally. Studying neurotrophins in relation to clinical variables revealed non-significant differences across the variables. Both neurotrophins were higher in females compared with males, although without statistical significance. BDNF and NT3 levels were lower in the presence of maternal chorioamnionitis, again without statistical difference. Detailed studies are, however, warranted to investigate further the changes in neurotrophin levels across these clinical parameters. NT3 levels did not change with gestational age like BDNF levels. However, NT3 levels show a trend similar to BDNF levels in infants who received antenatal steroids. If studied further, these findings may prove useful for future assessment of neuronal maturity and/or neurodevelopmental outcome.

Conclusion & Recommendation:

In conclusion, cord blood levels of BDNF may reflect the degree of neural maturity in premature infants. Increased BDNF and NT3 levels may also mediate improved neurodevelopmental outcome in infants who received antenatal steroids. It would be interesting to evaluate postnatal neurotrophin levels from premature infants. It may also be interesting to look at the significance of these levels in relation to various insults that would alter final neurodevelopmental outcome.

References: