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ORIGINAL ARTICLE

Serum Neuron-specific Enolase Levels in Preterm and Term Newborns and in Infants 1—3 Months of Age

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Background: Elevated serum levels of neuron-specific enolase (NSE) was initially assumed to be specific to neuronal tumors (particularly neuroblastoma), but is now known to accompany nontumoral conditions and tumors other than neuroblastomas. There is a need to establish normal ranges for NSE, especially in early infancy. The aims of this study were to determine reference values for NSE in newborns and young infants and to assess whether NSE levels in early infancy (i.e., preterm infants and term infants) differ from the adult reference range for this enzyme.

Methods: We enrolled 140 healthy babies, which included 40 preterm newborns (3—15 days old and born at 28—42 weeks gestation), 40 term newborns (< 1 month old and born at term), and 60 young infants 1—3 months old ($n = 20$ per subgroup of 1-, 2-, and 3-month-old infants). The determination of NSE levels was performed by the electrochemiluminescence immunoassay (ECLIA) method using the Elecsys 2010 device (Roche Diagnostics, Mannheim, Germany). The mean serum NSE levels for the preterm newborns was 21.83 ± 15.06 ng/mL [95% confidence interval (95%CI), 16.95—26.71 ng/mL]; term newborns, 18.06 ± 12.83 ng/mL (95%CI, 13.94—22.19 ng/mL); and young infants, 9.09 ± 4.38 ng/mL (95%CI, 7.96—10.23 ng/mL). The mean serum NSE level for infants 1—3 months old was within the ECLIA kit's normal range (4.7—18 ng/mL for adults), whereas the corresponding means for the preterm and term newborns were higher ($p < 0.001$, for both).

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Conclusion: Our findings suggest that adult reference values should not be applied to the preterm and term age groups.

1. Introduction

Tumor markers are substances that can be detected immunohistochemically on the cell surface or are secreted from tumor cells into blood, urine, and body fluids.[1,2] Neuron-specific enolase (NSE) is a glycolytic enzyme that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate. This enzyme is a 78-kD gamma-homodimer and is the dominant enolase-isoenzyme in neuronal and neuroendocrine tissues. The biological half-life of NSE in body fluids is approximately 24 hours.[2,3] Initial predominant isoforms of the enzyme are $\alpha\alpha$ dimers. Once cells begin to divide and migrate during embryological development, the $\alpha\alpha$ dimers are transformed into $\gamma\gamma$ dimers. Because increased levels of $\gamma\gamma$ dimers occur during neurogenesis and early neuronal differentiation, NSE is a good indicator of neuronal maturation and differentiation.[4] It is a marker for all types of neurons and for all neuroendocrine or paraneuronal cells. Initiation of NSE production occurs late in neural differentiation, which makes NSE a useful indicator of neural maturation.[2,5]

When neuronal damage occurs and/or the integrity of the blood—brain barrier becomes impaired, NSE is released into cerebrospinal fluid (CSF) and eventually into circulating blood. When NSE was first described, it was believed to be a specific marker for neuronal tumors, especially for neuroblastoma. Neurogenic tumors may indeed result in elevated blood levels of NSE; however, it has been shown that elevated NSE levels can also be associated with many other tumors and various clinical conditions.[6,7] Increased serum NSE may be detected in patients with conditions such as hypoxia after myocardial infarction, cell damage associated with the central and peripheral nervous system, subarachnoid hemorrhage, traumatic brain damage, Guillain—Barré syndrome, bacterial meningitis, encephalitis, sepsis, pneumonia, liver disease, and neonatal hyperbiluribinemia.[8—14]

Greater use of ultrasonography during the prenatal and newborn periods has increased the likelihood of detecting asymptomatic masses and very common neoplasms (including neuroblastoma) during these phases.[15] Adrenal masses smaller than 5 cm without metastases can be identified ultrasonographically during the intrauterine period and in premature babies, neonates, and in early infancy (< 3 months of age). However, such masses can also be detected through a combination of periodic ultrasound and biochemical testing for parameters such as vanillylmandelic acid (VMA), NSE, or ferritin.[16] For this reason, it is important to establish reference values for NSE in newborns and very young infants. The aims of this study were to determine reference values for NSE in newborns and young infants, and to assess whether NSE levels in early infancy (i.e., preterm infants and term infants) differ from the adult reference range for this enzyme.

2. Methods

The participants were 140 healthy preterm newborns or term infants younger than 3 months old who were examined between May 2010 and September 2010 in the Department of Neonatology and the Department of Pediatrics at Baskent University (Ankara, Turkey). The exclusion criteria were a low Apgar score (< 6 at 1 minute or at 5 minutes), a severe congenital abnormality, intraventricular hemorrhage, necrotizing enterocolitis, profound or suspected infection, development of infection within 3 days after serum sampling for the study, and pathological hyperbilirubinemia. The study was approved by the hospital's Ethical Research Committee. Written informed consent was obtained from the parents of each participant.

Each child's sex, gestational age, mode of delivery, birth weight, weight for gestational age, Apgar scores at 1 minute and 5 minutes, and serum bilirubin level were recorded. The serum NSE levels were also determined (see details later).

The babies were enrolled consecutively to meet target sample sizes for specific age groups that were categorized as follows: 40 preterm newborns (3—15 days old and born at 28—42 weeks gestation); 40 term newborns (< 1 month old and born at term), and 60 young infants 1—3 months of age (*n*20 per subgroup of 1-, 2-, and 3-month-old infants). Each preterm and term newborn underwent blood testing at least 72 hours after birth. Each baby in the young infant group had blood tested within the first 2 weeks of the month of life they were in at the time of the study.

Serum samples were collected and stored at −20°C until analysis. All samples were tested together after the target total sample size was reached. Testing for NSE was performed using a commercial electrochemiluminescence immunoassay (ECLIA) kit (Elecsys 2010 kit; Roche Diagnostics, Mannheim, Germany). Any infant with elevated serum NSE level, based on this kit's reference threshold (i.e., NSE > 30 ng/mL) was clinically evaluated and underwent repeat NSE testing, if necessary.

Descriptive statistics (i.e., mean values) and 95% confidence intervals (95%CIs) were calculated for each group and subgroup NSE findings. The Shapiro—Wilk test was used to assess whether variables were normally distributed. One-way analysis of variance (ANOVA) and the Tukey honest significant difference test were used to compare the mean ages for the three main study groups. The Student *t* test was used to compare the group means for other variables. Relationships between variables were tested using Spearman's *rho* correlation analysis.

3. Results

One baby with an elevated NSE level (96.21 ng/mL) had sepsis and had to be removed from the study. In total, 139

serum NSE values were evaluated (Table 1, Figure 1). The mean serum NSE levels for the preterm newborns was 21.83 ± 15.06 ng/mL (95%CI, 16.95–26.71 ng/mL); term newborns, 18.06 ± 12.83 ng/mL (95%CI, 13.94–22.19 ng/mL); and young infants, 9.09 ± 4.38 ng/mL (95%CI, 7.96–10.23 ng/mL). The mean serum NSE levels for the 1-, 2-, and 3-month-old subgroups of young infants were 8.92 ± 4.13 ng/mL, 7.63 ± 3.91 ng/mL, and 10.73 ± 4.70 ng/mL, respectively. The highest NSE level in each of the three main groups was 59.36 ng/mL (for preterm infants), 59.80 ng/mL (for term infants), and 21.40 ng/mL (for young infants). The highest NSE levels in each of the young infant subgroups were 16.80 ng/mL (for 1-month-old infants), 14.05 ng/mL (for 2-month-old infants), and 21.40 ng/mL (for 3-month-old infants).

The mean NSE levels of the preterm and term groups were significantly higher than the NSE levels in the young infants group ($p < 0.001$ for both). There was no significant association between the serum NSE level and the serum total bilirubin level or between the serum NSE level and birth weight ($p = 0.05$ for both; Figures 2A and 2B). The serum NSE level was negatively associated with Apgar scores at 1 minute and at 5 minutes (correlation coefficients $\rho = -0.228$ and $\rho = -0.246$, respectively; $p = 0.043$ and $p = 0.029$, respectively).

4. Discussion

Our main finding in this study was that the mean serum NSE level for infants 1–3 months old was within the ECLIA kit's normal range (i.e., 4.7–18 ng/mL for adults), whereas the corresponding means for the preterm and term newborns were higher. The 95%CI for the group of infants 1–3 months old was within the kit's reference range, whereas the 95% CIs for the preterm and term groups both exceeded the upper limit of this range.

Many factors may be associated with an elevation in the serum NSE level.[11,17–19] Hypoxia during the perinatal period is the most important factor, followed by intracranial hemorrhage and infection.[19–21] We investigated only healthy infants in our study; however, prematurity is linked with high morbidity and we therefore excluded preterm infants with intracranial hemorrhage, infection, and perinatal hypoxia.

Knitzel et al[22] investigated 192 healthy term newborns to establish reference values for NSE in cord blood. They excluded babies who had infections, hypoxia, and acidosis. Knitzel et al[22] observed that the median NSE was 8 ng/mL and the range for the 5th–95th percentile was 4.8–19.4 ng/ They concluded that cord blood NSE levels above this range in healthy term infants are associated with perinatal stress. The median serum NSE level for our 40 healthy term newborns was 14.53 ng/mL. Knitzel et al[22] evaluated cord blood (which likely reflect perinatal hypoxia), whereas we studied serum samples that were collected at least 72 hours after birth with the intent of eliminating the effects of perinatal hypoxia. We suspect that the effects of perinatal hypoxia persisted in our sample after birth and that the 72-hour period may have been insufficient to eliminate these effects. This is supported by our finding of a negative correlation between the NSE level and the Apgar score. Our observations suggest that increased NSE early after birth could be because of subclinical hypoxia.

Ekmektzoglou et al[23] studied adults with acute ischemic stroke and observed that serum NSE levels reached their maximum at 48 hours postevent. They postulated that the blood–brain barrier leakage caused high serum NSE levels after the stroke. In neonates and very young infants, the blood–brain barrier is immature and neuronal development is incomplete. Similar to the process postulated for stroke patients, it is possible that the status of the blood–brain barrier in newborns and very young infants could explain the broad range of serum NSE levels that we observed in our groups of healthy babies with no history of hypoxia.

A study of 112 healthy newborns and their mothers revealed that NSE levels in the neonates' umbilical arteries was in the range of 10–140 ng/mL and venous blood was in the range of 8.8–92 ng/mL.[24] On the 3rd day of life, venous blood samples were obtained from 18 neonates and the NSE levels ranged 11–200 ng/mL. The maternal NSE levels (range, 3–14 ng/mL) were lower than all neonatal levels that these authors observed in their study, which suggested independent fetal production of these proteins. The authors concluded that the high levels had not been caused by pre- or perinatal trauma or hypoxia because only healthy babies were investigated. A possible explanation for this is that immaturity of the blood–brain barrier permitted some passage of these brain-specific proteins without underlying damage. Another possible explanation is that the NSE measured was not of brain origin, but originated from different sources (e.g., thymus and fat tissue) during the fetal period. In our study, we observed that the preterm and term groups had significantly higher mean serum NSE than the commercial kit's reference range for adults.

Table 1 Serum neuron-specific enolase results for the study groups.

Group/subgroup	*n*	Serum NSE (ng/mL)					
		Mean ± SD	95% confidence interval	Minimum	Maximum	Median	Interquartile range
Preterm	39	21.83 ± 15.06	16.95–26.71	4.12	59.36	15.83	18.83
Term	40	18.06 ± 12.90	13.94–22.19	3.67	59.80	14.53	14.87
Young infants	60	9.09 ± 4.38	7.96–10.23	1.49	21.40	8.60	6.89
1 month old	20	8.92 ± 4.13	6.95–10.85	3.33	16.80	8.70	5.33
2 months old	20	7.63 ± 3.91	5.80–9.46	1.49	14.05	6.77	6.63
3 months old	20	10.73 ± 4.70	8.53–12.93	3.66	21.40	10.90	4.98

n = number; NSE = neuron-specific enolase; SD = standard deviation.

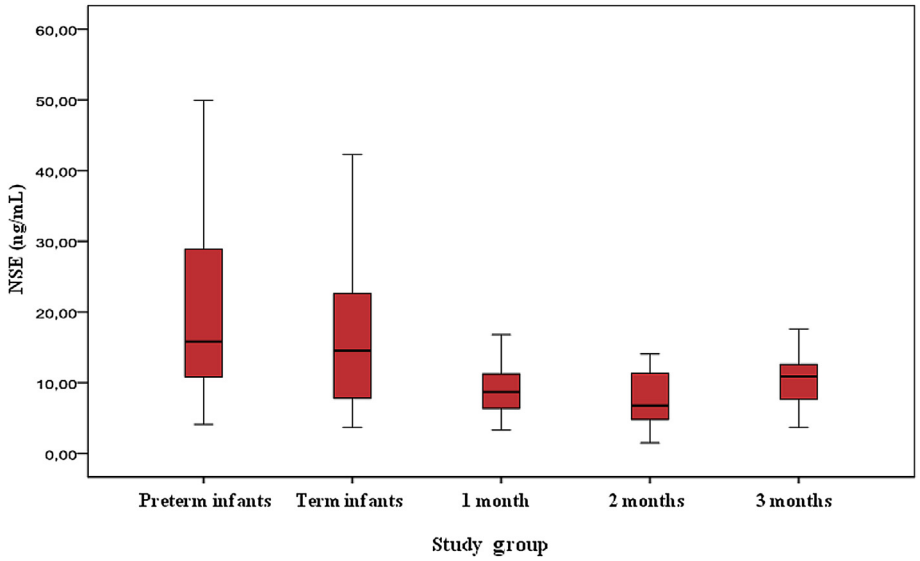

Figure 1 Serum neuron-specific enolase (NSE) levels in the study groups.

Furthermore, we found that this difference was most pronounced in the preterm babies. This may be related to factors such as heat exchange, inflation of the lungs, and oxygenation during perinatal adaptation.

In small premature babies, intraventricular bleeding alters NSE levels. Pellicer et al[25] observed the NSE level in the CSF of 39 preterm neonates (29.4 $\pm$ 2.2 gestational weeks) with known risk factors for brain damage. They documented brain damage by cerebral ultrasound and measured the NSE level by enzyme immunoassay. They found a CSF NSE level of 26.2 $\pm$ 8.1 ng/mL in babies with normal cerebral ultrasound and 144.8 $\pm$ 21.5 ng/mL in babies who had developed periventricular hemorrhage with paranchymal involvement during the 1st week of life. The increase in CSF NSE value was statistically significant ($p < 0.005$). They concluded that NSE is a marker for neuronal damage in the prenatal period. Birth hypoxia and intracranial hemorrhage are two potentially important factors for increased serum NSE levels. Our study only included babies with 1- and 5-minute Apgar scores > 6. However, this scoring method is subjective and birth hypoxia is more accurately assessed by analyzing cord blood gases at birth. We did not analyze cord blood gases and this was a limitation of our study. The routine practice at our hospital is that all babies who are born earlier than 32 weeks gestation undergo cranial ultrasonography. None had intracranial hemorrhage. We found no correlation between a high serum NSE level and intracranial hemorrhage.

High serum NSE levels have been detected in pediatric patients with septic shock.[26,27] Severe sepsis and septic shock lead to deficiencies of oxygen and glucose, which are necessary for brain development and normal neurological function. When oxygen and glucose are deficient, reactive oxygen radicals and cytokines are released and injure the developing brain. The resulting damage to the blood—brain barrier leads to a rise in the serum NSE level. In our study, we excluded babies with severe infection, but we did not exclude very small premature infants who were receiving

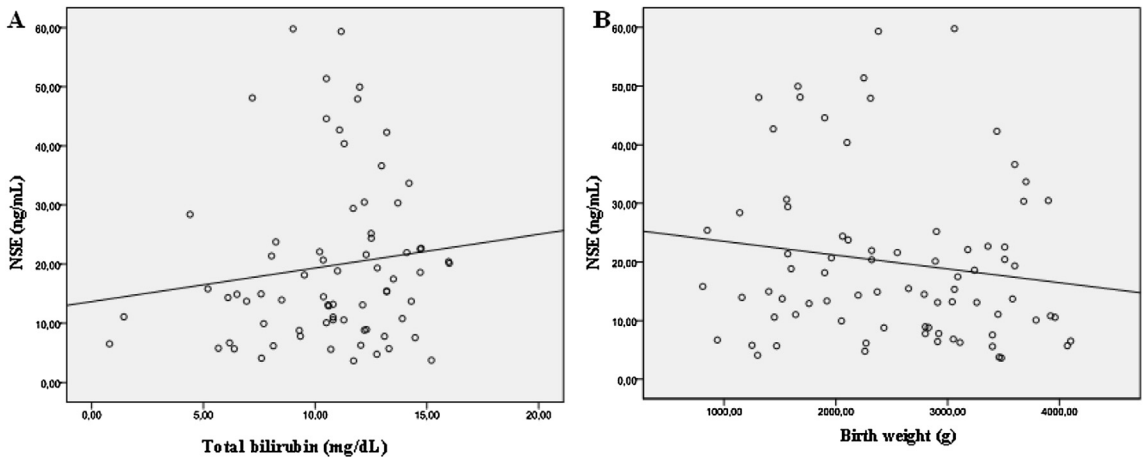

Figure 2 Relationships between serum neuron-specific enolase (NSE) level and total bilirubin (A) and between serum NSE level birth weight (B).

prophylactic antibiotics. Our findings of higher serum NSE levels in preterm and term newborns were not associated with sepsis or septic shock.

Akman et al[28] investigated correlations between an increased serum NSE level and serum bilirubin, and between increased an serum NSE level and auditory neuropathy. They observed no relationship between serum NSE and serum bilirubin level. The authors did find that infants with serum bilirubin $>$ 25 mg/dL had higher serum NSE values than infants with serum bilirubin levels $<$ 13 mg/dL. Serum bilirubin must attain this level because of gestational week and postnatal day to cross the blood—brain barrier and cause neuronal damage. Akman et al[28] concluded that the fact that this level was not exceeded explained why they found no statistically significant correlation between serum NSE and serum bilirubin. We also found no significant relationship between the serum NSE levels and serum bilirubin level.

In Turkey, various methods and laboratory kits have been used to measure serum NSE and reference values specific to each technique have been established.[29] It is important to apply the appropriate reference range for the method used. Our hospital employs the assay (i.e., an ECLIA kit) that we used in this study with a normal range of 4.7—18 ng/mL that is based on adult testing but is applied to all ages. The sample size for our study was limited; however, the results suggest that serum NSE levels in preterm and term newborns differ from those in adults. To our knowledge, the literature contains no reference values for serum NSE in preterm and term newborns. We observed ranges of 16.95—26.71 ng/mL in healthy preterm newborns and 13.94—22.19 ng/mL in healthy term infants. Our findings suggest that adult reference values should not be applied to these age groups. Further investigation is warranted with larger sample sizes and samples that include subgroups categorized by gestational week.

Conflicts of interest

The authors have no conflicts of interest relevant to this article to declare.

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