Original Article

Subclinical hypoxia of infants with intrauterine growth retardation determined by increased serum S100B protein levels

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Aim: To test the hypothesis that serum S100B levels could be useful in detecting neurological damage in infants with intrauterine growth retardation (IUGR).

Materials and methods: The study group consisted of infants with IUGR and the control group consisted of age-matched healthy infants. S100B protein levels were measured after birth and compared between groups.

Results: For this study, 43 infants with IUGR and 25 infants as a control group were recruited. Gender, gestational age, type of delivery, and maternal age of the groups were statistically insignificant, with the exception of the mean birth weights (2120 ± 450 g in the IUGR group and 3096 ± 570 g in the control group (P < 0.001), respectively). S100B protein levels of the IUGR infants (1.13 ± 0.54) were significantly higher than those of the control group (0.45 ± 0.13) (P < 0.001). IUGR infants treated with antenatal steroids showed lower S100B levels than IUGR infants that did not receive antenatal steroid treatments (P < 0.05). The study group infants were divided into 2 groups, for growth retardation (GR) that was asymmetric (n = 15) and symmetric (n = 28). The asymmetric and symmetric GR infants' S100B levels were 1.14 ± 0.47 pg/mL and 1.21 ± 0.34 pg/mL, respectively, and no significant differences were found between the 2 groups in terms of S100B levels (P = 0.32).

Conclusion: The results of this study favor the opinion that there is an existing intrauterine hypoxia causing hypoxic brain tissue damage in IUGR infants, even when followed up with modern obstetrical screening protocols. Measurements of S100B may be useful in the prediction of outcome in these infants.

Key words: S100B, IUGR, intrauterine growth retardation, intrauterine hypoxia

İntrauterin büyüme gerelikli infantların subklinik hipoksilerinin artmış serum S100B protein seviyeleri ile saptanması

Amaç: Bu prospektif çalışmada intrauterin gelişme geriliği (IUGR) olan infantlarda nörolojik hasarı tespit etmede S100B seviyelerinin faydalı olduğu hipotezi test edildi.

Yöntem ve gereç: Çalışma grubu İUGR infantlardan ve kontrol grubu benzer yaştaki sağlıklı infantlardan oluşmaktaydı. S100B protein seviyeleri doğum sonrası ölçüldü ve 2 grup karşılaştırıldı.

Bulgular: Kırk üç IUGR ve kontrol grubunda 25 infant çalışmaya alındı. Cinsiyet, gestasyonel yaş, doğum şekli ve grupların maternal yaşı arasında ortalama doğum ağırlığı (sırasıyla IUGR grubunda 2120 ± 450 g kontrol grubunda

3096 ± 570 g (P < 0,001)) haricinde istatistiksel farklılık yoktu. S100B protein seviyeleri IUGR infantlarda (1,13 ± 0,54) kontrol grubundan (0,45 ± 0,13) anlamlı derecede daha yüksek bulundu (P < 0,001). S100B seviyelerinin antenatal steroid tedavisi alan IUGR infantlarda antenatal steroit tedavisi almayan İUGR infantlara göre daha düşük düzeyde olduğu gösterildi (P < 0,05). Çalışma grubundaki infantlar asimetrik (n = 15) ve simetrik (n = 28) gelişme geriliği olmak üzere 2 gruba ayrıldılar. Asimetrik ve simetrik gelişme geriliği olan infantların S100B seviyeleri sırasıyla 1,14 ± 0,47 pg/mL ve 1,21 ± 0,34 pg/mL idi. İki grup arasında S100B seviyesi bakımından anlamlı farklılık bulunmadı (P = 0,32).

Sonuç: Bu çalışmanın sonuçları modern obstetrik metodlarla takip edilse de, İUGR infantlarda hipoksik beyin hasarına neden olan intrauterin hipoksi varlığı düşüncesini desteklemektedir. Bu proteinin ölçülmesi bu infantların akibetlerini tahmin etmede yaralı olabilir.

Anahtar sözcükler: S100B, IUGR, intrauterin gelişme geriliği, intrauterin hipoksi

Introduction

Placental insufficiency, with a consequent reduction in fetal nutritive and oxygen supply, is one of the most important causes of intrauterine growth restriction (IUGR) and fetal hypoxia, which is one of the main causes of perinatal mortality and morbidity (1,2). Episodes of acute and chronic fetal hypoxia can result in brain lesions, which may leave long-term neurological sequelae such as auditory and visual impairment, mental retardation, and seizure disorders. Moreover, minimal brain alterations, such as a reduction in learning capabilities, dyslexia, and attention-deficit/hyperactivity disorder, could originate from the prenatal and/or perinatal exposure to hypoxia (2-4). Nowadays, the management of IUGR includes the combination of serial morphological ultrasound examinations to assess fetal growth and amniotic fluid quantity, combined with functional testing such as a biophysical profile, fetal heart rate testing, and Doppler examinations of fetal hemodynamics (5). Unfortunately, even if hypoxia is detected, none of the presently available tests can define the amount of hypoxia that the fetus can endure without developing hypoxic brain lesions or the length of duration. Only the answers to these questions would enable the definition of the optimal timing of delivery in the case of chronic fetal hypoxia and prevention of perinatal brain damage. Although in this situation the delay of the delivery might cause the prolongation of fetal exposure to hypoxia, which could affect brain development and cause brain damage, early delivery carries the risk of prematurity and associated risk of neurological disorders. Additionally, IUGR infants may show hyperreflexia, hyporeflexia, jitteriness, and/or hypertonicity in their early life. In most situations, neurological examinations of IUGR infants are uneventful and there is no evidence for the prediction of long-term neurological disorders in these babies (3,4,6). Therefore, the ability to monitor high-risk fetuses in the perinatal period by use of biochemical indexes could be particularly useful in detecting cases at risk of adverse neurological outcomes, and in determining the timing of insults that damage the central nervous system as early as possible with respect to future measures of prevention.

S100B is an acidic calcium-binding protein that is released by brain tissue and is known to be highly specific to the nervous system (7). It is thought to play a dual role in the regulation of cell function, being beneficial to cells at low doses but detrimental at high doses. The protein appears to be most abundant in glial cells, although its presence in neuronal subpopulations has also been reported. These findings have answered the question about the putative role of S100B in brain injury (8). Therefore, increased S100B concentrations in biological fluids (e.g. cerebrospinal fluid, blood, urine, and amniotic fluid) of adults, infants, and fetuses after cell injury in the nervous system have supported the use of S100B as a biochemical marker of brain damage (7). It was found that this protein is increased in demyelinating brain diseases, phenylketonuria, and especially in hypoxic-ischemic brain damage (HI) (7,9). S100B in the blood was measured, hypothesizing that during active brain injury, the protein could be released from the damaged tissue and part of it could spread into the systemic circulation, also as a result of the hemodynamic rearrangement of the blood-brain barrier. In this regard, increased

concentrations of S100B were detected 48-72 h before any clinical, laboratory, or ultrasonographic signs of intraventricular hemorrhage in preterm newborns and of hypoxic ischemic encephalopathy (HIE) in full-term newborns (8). Currently, there are few studies about S100B in the serum of infants with IUGR that are under risk of perinatal brain damage (10-12).

In the present study, we aimed to investigate the serum S100B levels in infants with IUGR to provide early prediction of possible negative neurodevelopmental outcomes, for both early and later life in this particular population.

Materials and methods

The study was performed at 2 different tertiary referral centers for obstetrics and neonatal intensive care units from February to July of 2007. We studied 43 women with singleton pregnancies complicated by IUGR, between the 30th and 42nd weeks of gestation. Informed consent was obtained from both hospitals' ethics committees and the parents. Gestational age was determined by clinical data and by a first trimester ultrasound scan. IUGR was defined by the presence of ultrasonographic signs (biparietal diameter below the 10th percentile and abdominal circumference below the 5th percentile) according to the nomograms of Campbell and Thoms (7,13). A drop in the percentile of fetal sizes was recorded between the first scan after referral and the final scan before delivery. Fetal growth restriction was confirmed by a birth weight below the 10th percentile in all of the fetuses. The ponderal index was calculated for each of the GR infants. Afterwards, infants were divided into 2 groups related to the ponderal index: asymmetric GR infants (<50% of ponderal index) and symmetric GR infants (>50% of ponderal index) (14). The control group consisted of 25 normal fetuses, matched for gestational age at sampling (range: 30-42 weeks of gestation) and birth weights between the 10th and 90th percentiles.

All women, in both the study and the control groups, gave birth either vaginally or by elective cesarean section, based on obstetric indications. At delivery, the umbilical cord was clamped before any signs of breathing were seen and blood was drawn from the umbilical vein for the standard assessments (blood cell count, alanine aminotransferase, aspartate aminotransferase, blood urea, creatinine, glucose, electrolytes, interleukin-6, and C-reactive protein).

Infants that fulfilled all of the following criteria were admitted to the study: no maternal illness, no signs of fetal distress, a pH greater than 7.2 in cord blood or venous blood, and Apgar scores above 7 at 1 and 5 min. Exclusion criteria were multiple pregnancies and maternal alcohol or cocaine addiction, infants with any malformation, cardiac or renal diseases, hemolytic disease, or intrauterine infections.

Peripheral venous blood samples for S100B measurement were obtained from a peripheral vein 12 h after birth and centrifuged immediately at 5000 g for 10 min, and the sera were stored at –80 °C until concentration analyses were performed. All serum samples for S100B were of good quality without hemolysis. The S100B serum levels were determined by fully automatic electrochemoluminometric immunoassay (Elecsys S100®; Roche Diagnostics, Penzberg, Germany) with a measurement range of 0.005-39 ng/L.

A neurological examination was performed at the same time as the blood sampling and during hospital discharge. Neonatal neurological conditions were classified as normal or abnormal. An infant was considered to be abnormal when one or more of the following neurological syndromes were present: hypo- or hyperkinesias, hyper- or hypotonicity, dystonia, hemisyndrome, apathy syndrome, or hyperexcitability syndrome.

Statistical analysis

For evaluation of the distribution of S100B measurement results, histogram and Kolmogorov-Smirnov tests were applied. Student's t-test was used for statistical evaluation of continuous variables. Relationships between S100B protein levels and birth weight, birth length, maternal age, and gestational age were evaluated with Pearson's correlation analysis. The gender of the infants, type of birth, and type of anesthesia were compared to each other using the chi-square test. Data were analyzed with SPSS version 13.0 (SPSS Inc., Chicago, USA) with significance defined as $P < 0.05$.

Results

The findings of the study are summarized in the Table. The study group consisted of 43 infants and the control group of 25 infants. No significant differences between the 2 groups were found in terms of gender, gestational age at delivery, type of delivery, antenatal glucocorticoid treatment, and maternal age, with the exception of birth weight. The birth weights of the 2 groups were 2120 ± 450 g in the IUGR group and 3096 ± 570 g in the control group (P < 0.001), respectively. There were no significant differences in the laboratory parameters (red blood cell count, alanine aminotransferase, aspartate aminotransferase, blood urea, creatinine, glucose, electrolytes (sodium, potassium, and calcium), interleukin-6, and C-reactive protein) between the IUGR and control groups upon admission to the neonatal intensive care unit (P > 0.005). The study and the control group were found to be similar with respect to neurological examinations. In both groups, none of the infants showed neurological abnormalities at the time of discharge from the hospital, and no overt neurological syndromes were observed during recovery. Isolated and transient symptoms, including hypertonia/hypotonia, dystonia, and hyperexcitability, were shown in 15 of the IUGR infants and in 11 of the control infants. Mean fetal S100B levels in the IUGR group (1.13 ± 0.54 pg/mL) were significantly higher (P < 0.001) than those in the control group (0.4 ± 0.13 pg/mL). There was a weak and low correlation between gestational age and S100B protein levels (r = 0.24, P = 0.12) and a weak and low correlation between birth weight and S100B protein levels (r = −0.2, P = 0.006). When the IUGR fetuses were grouped according to antenatal steroid treatment, status of neurological examination, type of delivery, and type of anesthesia during delivery, those groups were found to be similar with respect to these parameters, except for the presence of antenatal steroid treatment. IUGR infants treated with antenatal steroids showed lower S100B levels than those without antenatal steroid treatment (P < 0.05). The study group infants were divided into 2 groups: asymmetric GR and symmetric GR. The asymmetric GR group consisted of 15 neonates, and the symmetric SGA group consisted of 28 neonates. The asymmetric and symmetric GR infants' S100B levels were 1.14 ± 0.47 pg/mL and 1.21 ± 0.34 pg/mL, respectively, and no significant differences were found between the 2 groups in terms of S100B levels (P = 0.32).

Table. Characteristics of the study population and comparison of intrauterine growth retardation (IUGR) and control groups.

	IUGR (n = 43)	CONTROL (n = 25)	P
Maternal age (mean ± SD, years)	28.4 ± 4.0	28.8 ± 4.5	0.7
Gestational age at delivery (mean ± SD, weeks)	37.7 ± 2.8	38.0 ± 2.5	0.6
Gender (male/female)	22/21	14/11	0.7
Birth weight (mean ± SD, g)	2120 ± 450	3096 ± 570	<0.001
Maternal glucocorticoid treatment (N/%)	11/25	5/20	0.5
Type of delivery (cesarean/vaginal)	26/17	13/12	0.5
Type of anesthesia during delivery (general/regional)	23/20	15/10	0.6
Neurological examination (normal/abnormal)	28/15	14/11	0.9
S100B protein (pg/mL)	1.13 ± 0.54	0.45 ± 0.13	<0.001

Discussion

The present study showed that S100B levels increased in infants with IUGR at birth, and asymmetric and symmetric GR infants had similar S100B levels. Additionally, IUGR newborns with an abnormal neurological outcome had similar S100B levels as infants with IUGR and an uneventful neurological follow-up. The present study was not the first to report the increased level of S100B protein in IUGR infants. Previous human studies showed that the continuous release of the protein from damaged nervous tissue was the main cause of the high level of S100B in blood obtained from preterm and term infants with IUGR, due to chronic hypoxia (7,10,12,15). Additionally, animal studies have shown that intrauterine hypoxia is associated with persistent elevations of S100B protein in plasma, which supports S100B as a useful early marker of fetal hypoxia (16). It was also shown that S100B protein levels in maternal and umbilical blood were increased in IUGR fetuses and represented cerebral cell damage in the perinatal period (17).

Although S100B is a protein mainly concentrated in the central nervous system, it was shown that pathological conditions that develop during pregnancy affect the S100B level in the placenta, umbilical cord tissue, fetal membranes, amniotic fluid, and the fetal cord blood (17). Therefore, biochemical markers in the umbilical vein blood also reflect placental function. The half-life of this protein is approximately 1 h (4). Therefore, placental production might affect the serum levels of the protein in the early hours of life, but is obviously not responsible for the high levels found at later points in time. It is suggested that elevated S100B levels 12 h after birth are a useful tool for the early detection of neurological events in infants (18). In this regard, in this study, blood samples were obtained from a peripheral vein 12 h after birth to measure the accurate levels of S100B and to exclude placental contributions.

These findings, and the evidence that blood levels of S100B were particularly high in IUGR newborns with and without abnormal short-term neurological outcomes, together suggest that the level of S100B may reflect the extent of brain damage after intrauterine hypoxic insult. The lack of difference in S100B levels between IUGR infants with normal and abnormal short-term neurological outcomes supports the concept that the neurological symptoms observed in the first few months after birth in preterm infants who will develop cerebral palsy are neither sensitive nor specific enough to ensure reliable prognoses. Irritability, abnormal finger posture, spontaneous Babinski reflex, weakness of the lower limbs, transient abnormality of tone, and a delay in achieving motor milestones are some of the neurological signs that have been described in these high-risk preterm infants (6). All of these symptoms may be encountered before the onset of cerebral palsy or during "transient dystonia," dissociated motor development, and other transient neurological disturbances, which disappear during the first or second year of life. Moreover, no correlations have been found among any of these symptoms and the severity of future motor impairment (6). Therefore, a traditional neurological examination fails to predict the development and severity of long-term neurological abnormalities.

Antenatal glucocorticoids are widely used in premature infants, where they reduce neonatal morbidity and mortality. IUGR fetuses are at risk of preterm delivery and thus are likely to receive antenatal glucocorticoids to promote lung maturation. Because glucocorticoids alter vascular tone, it was questioned whether such treatment may induce fetal cardiovascular alterations in IUGR infants; some authors claimed that the fetal cardiovascular response to maternal betamethasone administration in IUGR fetuses is significantly different than that seen in healthy fetuses (19). The IUGR fetus displays rebound cerebral perfusion that is not seen in the healthy fetus, which is associated with increased oxidative stress and apoptosis in the fetal brain. Therefore, maternally administered glucocorticoids may have deleterious effects on the brain of IUGR fetuses. In light of this fact, it is logical to expect a higher level of S100B in steroid-administered groups, but our results contradicted this hypothesis rather than supporting it.

To date, there have been no human studies that evaluated the relationship between antenatal steroid administration and S100B protein blood level in IUGR infants. However, there was one animal study

that investigated the effects of a single course of antenatal betamethasone on S100B concentrations in preterm rats, in which a clinically equivalent dose and half of this dose was used (19). The authors found that the hippocampal S100B content was reduced by a clinically equivalent dose of 12 mg twice, but not by half of this dose. Interestingly, the effect of betamethasone on brain cell proliferation also showed a dose-dependent pattern, as investigated in the same hippocampal homogenates. The authors speculated that lowering the dose of antenatal steroids may be less detrimental for brain maturation.

Conclusion

The results of this study and previous studies warrant consideration and favor the opinion that there is an existing intrauterine hypoxia causing hypoxic brain tissue damage in IUGR infants, even when followed up with modern obstetrical screening protocols. The major goals of modern obstetric screening procedures are the identification of growth-restricted fetuses among the small-for-gestational-age fetuses. Early detection of fetal hypoxia in high-risk pregnancies and timely termination of high-risk pregnancies will prevent the development of hypoxic brain injury.

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