S100B Protein Levels in Cord Blood: A Study in Normal and Growth-Retarded Fetuses

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Abstract:
Follow-up studies have shown that the vast majority of neurological abnormalities present during childhood can have a prenatal or perinatal origin. It is relevant, therefore, to investigate the timing of adverse insults in the search for measures of prevention. However, such knowledge is still incomplete and subject to debate. Until recently, clinical-laboratory assessment was based essentially on biochemical aspecific parameters, ultrasound and Doppler patterns, and the determination of blood pH and gases. However, the measurement of brain constituents may offer a direct indicator of cell damage in the nervous system. The S100B protein, a calcium-binding protein highly concentrated in the nervous system, appears to meet the criteria required of such a marker in prenatal and perinatal medicine for its reproducible, simple and sensible measurements. The aim of this study was to determine whether S100B protein could be helpful in the detection of brain distress in intrauterine growth-retarded (IUGR) fetuses, we studied, by a case-control study, the correlation between S100B protein and the degree of fetoplacental blood flow impairment. Maternal and umbilical blood samples were collected at delivery from IUGR pregnancies with normal (n=10) or abnormal (n=10) umbilical artery Doppler findings and from 20 uncomplicated pregnancies. S100B protein levels were measured by means of a specific RIA and flow velocimetry waveforms were recorded from uterine, umbilical, and fetal middle cerebral arteries. The results showed that maternal plasma S100B protein levels were under the limit of detection in both IUGR and control groups. In contrast, fetal S100B protein levels in umbilical plasma were significantly higher (p=0.04) in IUGR patients (123.5±69.7 fmol/mL) compared to the controls (52.4±20.2 fmol/mL). IUGR fetuses with redistribution of blood flow showed the higher concentration of the protein (164.8±54.9 fmol/mL). Fetal S100B protein levels correlated negatively with middle cerebral artery pulsatility index (r= -0.528; p=0.02), and positively with umbilical artery pulsatility index to middle cerebral artery pulsatility index ratio (r=0.489; p=0.03). In the control group, a negative significant correlation between S100B protein and gestational age was found(r = -0.76; p= 0.01).

Conclusion: Circulating S100B protein is increased in IUGR fetuses and correlates with cerebral hemodynamics, suggesting that it may represent an index of cerebral cell damage in the perinatal period.

Abbreviations:

Introduction:
The S100 family of calcium-binding proteins, first isolated in 1965 by Moore,7 in a subcellular fraction from bovine brain, contains approximately 16 members, each of which exhibits a unique pattern of tissue- or cell type–specific expression. Although the distribution of these proteins is not restricted to the nervous system, the involvement of several members of this family in nervous system development, function, and disease has sparked new interest in these proteins. S100B protein, one of the original two members of this family, is an acidic calcium-binding protein with a molecular weight of 21 kD. It is present extracellularly, intracellularly, and in the cytosol; its half-life is approximately 2 h, and it is mainly eliminated by the kidney.2 S100B protein is present in central nervous system and is concentrated in the glial cells, astrocytes, Schwann cells, and neurons.3,4 It regulates several cellular functions (cell-cell communication, cell growth, cell structure, energy metabolism, contraction, and intracellular signal transduction). Elevated plasma levels are found in patients with brain damage.4 Abnormal S100B protein levels have been associated with neurobehavioral abnormalities,5 and microcephaly caused by in utero cocaine exposure,6 and abnormal S100B protein
immunoreactivity cells in anencephalic fetuses have been shown.7 The S100B protein concentration in blood and in CSF is increased as result of brain damage in adults and infants.8,9,11 Although S100B protein is detectable in the umbilical cord blood of preterm and term fetuses,12,13 increased circulating protein levels have been related to the occurrence of intraventricular hemorrhage in preterm infants.14

IUGR is commonly accepted as an expression of persistent suppression of genetic growth potential caused by decreased oxygen and substrate supply.15 IUGR is generally associated with uteroplacental blood flow insufficiency (up to 50%) because of impaired trophoblast invasion of spiral arteries, which are not transformed to low resistance vessels.16 Feto placental insufficiency, and subsequent fetal hypoxia, activates a cascade of pathophysiologic events leading to brain damage in which vasoactive agents and calcium-mediated effects are involved.17 The aim of this study was to estimate the cord blood levels of S100B protein in IUGR fetuses compared with levels in normally grown fetuses, and to correlate the levels of S100B protein with hemodynamic findings, to evaluate its potential role as an indicator of cerebral cell damage.

Subjects and Methods:

This study was performed in the Departments of Gynecology and Obstetrics, and of Pediatrics of Al-Minya University Hospital during the period between January 2004 and July 2004. We studied 20 women with singleton pregnancies complicated by IUGR between 28 and 40 wk of gestation. 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,18 and a fall in the percentile of fetal sizes was recorded between the first scan after referral and the final scan before delivery. IUGR was confirmed at birth if the neonatal weight was below the 10th percentile.19

FVWs of the main branch of the uterine artery bilaterally, umbilical artery, and fetal middle cerebral artery were recorded by means of a duplex pulsed color Doppler ultrasound (TOSHIBA SSA340A, Tokyo, Japan), with a convex 3.75-MHz transducer, and the RI (peak systolic velocity - end-diastolic velocity / peak systolic velocity) and PI (peak systolic velocity - end-diastolic velocity / mean velocity) were calculated automatically by the built-in software. Doppler waveforms were obtained in the absence of fetal body or breathing movements. In every record, three to five consecutive cardiac cycles were examined, and the mean of at least three values from each vessel was used for subsequent analysis. Abnormal RI for uterine artery or PI for umbilical artery was defined as >95th percentile for gestational age for uncomplicated pregnancies.20 Similarly, an abnormal middle cerebral artery PI <5th percentile for gestational age for uncomplicated pregnancies and an umbilical artery PI to middle cerebral artery PI ratio >1 were considered as indexes of redistribution of fetal blood flow.21

The control group consisted of 20 normal fetuses matched for gestational age at sampling (range, 32–40 wk of gestation) and birth weights between the 10th and 90th percentiles.19 In these pregnancies the FVWs of the fetal middle cerebral arteries were not recorded because velocimetry waveforms in the uterine and umbilical arteries were normal. All subjects were delivered by elective cesarean section, performed within 1 h after FVW recording. Maternal plasma was collected from cubital vein before induction of anesthesia. At delivery, the umbilical cord was clamped and blood was drawn from the umbilical vein. None of the patients experienced uterine contractility before cesarean section. Indications for elective cesarean section in the controls included breech presentation, previous cesarean section, placenta praevia, and maternal cardiac disease. Directly after birth, every newborn was given a thorough physical examination by the pediatrician. Apgar scores at one, and five minutes were recorded.

**S100B protein measurement:**

Heparin-treated blood samples taken at birth were immediately centrifuged at 900 x g for 10 min, and the supernatants stored at -70°C. The S100B protein concentration was measured in all samples using a commercially available RIA kit (Sangtec 100, AD Sangtec Medical, Bromma, Sweden).specific to the B-subunit of the protein, which is known to predominate (80–96%) in the human brain, as reported.4,11 Each measurement was performed in duplicate according to manufacturer’s recommendations, and the averages were reported. The sensitivity of the assay was 0.2 µg/L (fmol/mL). The inter- and intrassay coefficient of variation was 10% and <5%, respectively. The assay detects exclusively S100B protein and no cross-reactivity has been found with other S100 proteins.

**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. Chi-square test was used to compare between two groups as regards non-parametric data.
4. ANOVA was used for comparison between more than two groups.
5. Pearson correlation was used to correlate two quantitative variables.

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

Results:

Table I: Comparison between the IUGR group and the control group.

<table>
<thead>
<tr>
<th></th>
<th>IUGR (n=20)</th>
<th>Controls (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>25.6±3.5</td>
<td>26.5±4.2</td>
<td>0.466</td>
</tr>
<tr>
<td>Gestational age at sampling (wk)</td>
<td>34.5±2.5</td>
<td>35.4±3.1</td>
<td>0.318</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1650±510</td>
<td>2420±420</td>
<td>0.001*</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>330.2±110.3</td>
<td>445.1±122.2</td>
<td>0.003*</td>
</tr>
<tr>
<td>Apgar 1 min &lt;7</td>
<td>3(15%)</td>
<td>1(5%)</td>
<td>0.548</td>
</tr>
<tr>
<td>Apgar 5 min &lt;7</td>
<td>2(10%)</td>
<td>0(0%)</td>
<td>0.437</td>
</tr>
<tr>
<td>Uterine artery RI</td>
<td>0.67±0.03</td>
<td>0.55±0.09</td>
<td>0.03*</td>
</tr>
<tr>
<td>Uterine artery RI &gt; 95th percentile</td>
<td>12(60%)</td>
<td>0(0%)</td>
<td>0.00003*</td>
</tr>
<tr>
<td>Umbilical artery PI</td>
<td>1.42±0.21</td>
<td>0.8±0.18</td>
<td>0.001*</td>
</tr>
<tr>
<td>Umbilical artery PI &gt;95th percentile</td>
<td>10(50%)</td>
<td>0(0%)</td>
<td>0.0002*</td>
</tr>
<tr>
<td>MCA PI</td>
<td>1.4±0.24</td>
<td>not recorded</td>
<td></td>
</tr>
<tr>
<td>MCA PI &lt;5th percentile</td>
<td>10(50%)</td>
<td>not recorded</td>
<td></td>
</tr>
<tr>
<td>Umbilical artery PI/MCA PI</td>
<td>1.01±0.33</td>
<td>not calculated</td>
<td></td>
</tr>
<tr>
<td>Umbilical artery PI/MCA PI &gt;1</td>
<td>10(50%)</td>
<td>not calculated</td>
<td></td>
</tr>
<tr>
<td>Maternal plasma S100B (fmol/mL)</td>
<td>undetectable</td>
<td>undetectable</td>
<td></td>
</tr>
<tr>
<td>Fetal plasma S100B (fmol/mL)</td>
<td>123.5±69.7</td>
<td>52.4±20.2</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

*p<0.05 is significant.

In both groups, none of the infants showed neurologic abnormalities at the time of discharge from the hospital, and no overt neurologic syndromes were observed during recovery. Isolated and transient symptoms in 10 IUGR infants, including hypertonia-hypotonia (n=7), and hyperexcitability (n=3) have been shown.

Maternal plasma S100B protein levels were under the limit of detection in both IUGR and control groups. In contrast, fetal plasma S100B protein levels in the IUGR group (123.5±69.7 fmol/mL) were significantly higher (p=0.04) than those in the control group (52.4±20.2 fmol/mL) and correlated negatively with middle cerebral artery PI (r=-0.528; p=0.02)(figure 1),
and positively with the umbilical artery PI to middle cerebral artery PI ratio (r=0.489; p=0.03)(figure 2). Although plasma S100B protein levels were higher at greater umbilical artery PI, this correlation was not statistically significant (r=0.23; p=0.27). When IUGR fetuses were grouped according to the presence of redistribution of fetal blood flow (middle cerebral artery PI <5th percentile and the umbilical artery PI to middle cerebral artery PI ratio >1), plasma S100B protein levels were significantly higher (p=0.001) only in those fetuses with redistribution of fetal circulation (164.8±54.9 fmol/mL). In IUGR fetuses without this brain-sparing effect, plasma S100B protein levels(81.1±59.6 fmol/mL) were not different from those found in the controls( 52.4 ± 20.2 fmol/mL) (p=0.241).

In the control group, a negative significant correlation between S100B protein and gestational age was found (r= -0.76; p=0.01), whereas this correlation was lost in the IUGR group. No correlation was found between S100B protein and birth weight in any group.

Discussion:
The present research shows that S100B protein is present in considerable amount in the fetal circulation, whereas it is undetectable in plasma of pregnant women.

Amer-Wahlin et al.,22 also reported that S100B protein can be detected in cord blood of healthy newborns[arterial 1.10 (0.38-5.50) µg/L; venous 0.98 (0.43-2.70) µg/L]. A new finding in their study was that, in contrast with S100B protein measurements in adults,23 hemolysis can affect measurements in neonates, increasing the concentration. In this study no macroscopic hemolysis was observed. Maschmann et al.,24 measured serum S100B protein in blood of healthy newborns (range between 2.5 and 97.5 centile: 0.66–3.33 µg/L). However, blood was collected at no fixed time points but over several days after birth. This makes it difficult to compare the results with those of our study because S100B protein levels may change in the days after birth.

Concentrations of S100B protein in amniotic fluid increase from the 15th week of gestation to the 18th week from 0.45 µg/L to 0.58 µg/L.25 S100B protein expression in the developing brain of the newborn is much higher than it is in adults.26 These factors may account for raised levels of S100B protein in cells of the central nervous system in newborns compared with adults. The fact that S100B protein is found in cord blood in this study is not explained by these factors.

For S100B protein to appear in peripheral blood, two things have to happen. Firstly, S100B protein must be released into the extracellular matrix and from there to the CSF. It is believed that only damage to cells containing S100B protein could account for the appearance of S100B protein in CSF, because in normal circumstances, S100B protein is not found in abundance in the extracellular matrix surrounding cells containing S100B protein. If this is true, why is S100B protein detected in healthy newborns without any neurological problems? Perhaps the S100B protein content of the extracellular matrix is much higher in newborns. Secondly, S100B protein can only reach the peripheral blood by traversing the blood-brain barrier. Gazzolo et al.,24 have postulated that, in newborns, the blood-brain barrier may be immature and therefore more permeable to S100B protein than in adults. This may be one explanation for the raised S100B protein levels in peripheral blood in newborns, if the source of this S100B protein is indeed located primarily in the central nervous system. S100B protein has been detected in various other tissues, although the total amount is small compared with that found in the central nervous system.24 Minute amounts have been detected in chondrocytes of adults as well as in fetal cartilage.27 S100B protein from other tissues may be an, additional, explanation for the S100B protein found in peripheral blood in healthy newborns. The only way of determining the source of the S100B protein measured in healthy newborns is to simultaneously determine the S100B protein content of CSF and peripheral blood in these newborns; this is obviously ethically unacceptable. Therefore we have to use CSF obtained in the course of the treatment of neurological disorders. It is possible that the origin of some of the S100B protein in cord blood is the placenta, but Wirds et al.,13 reported that this seems unlikely because the arterial cord blood S100B protein levels were higher than the venous cord blood S100B protein levels in their study.

In this study, umbilical cord blood levels of S100B protein have been shown to be inversely correlated with gestational age in healthy newborns, suggesting a neurotrophic role for this protein in the third trimester of pregnancy. A similar finding was also observed by Gazzolo et al.12

In our study, we showed that S100B protein levels were higher in growth-retarded fetuses with redistribution of blood flow and correlated with the degree of fetal hemodynamic impairment as indicated by middle cerebral artery PI and umbilical artery to middle cerebral artery PI ratio; our observed results are comparable to those of Gazzolo et al.28 In contrast, IUGR fetuses without redistribution of blood flow showed S100B protein levels similar to those found in fetuses with normal growth. Thus impairment of fetal growth per se does not affect S100B protein concentrations in the fetal circulation, as also...
demonstrated by lack of correlation between protein levels and birth weight.

Although in our series neurologic outcome, at least at the time of hospital discharge, was normal, we hypothesize that increased S100B protein levels in IUGR fetuses with redistribution of fetal circulation may reflect fetal cellular brain damage owing to chronic hypoxia. In the last decade, several studies demonstrated that Doppler findings, and particularly the ratios of Doppler FVWs in cerebral and peripheral vessels, are reliable indices of redistribution of fetal blood flow and are correlated with the degree of fetal hypoxia and perinatal outcome. In IUGR pregnancies with impaired placental perfusion, transfer of oxygen and nutrients from the mother to the fetus is reduced, leading to a cardiovascular response characterized by a redistribution of cardiac output to maintain oxygen supply to the brain, heart, and adrenal at the expense of visceral organs to preserve their function. However, despite this hemodynamic mechanism, adverse effects of hypoxemia on brain maturation have been demonstrated in clinical and histological studies. Disturbances in the development of fetal behavioral states, defined as expression of brain injury, have been reported and ultrastructural studies in human and animal have shown that chronic intrauterine stress affected the functional maturation of various organ systems, including fetal brain. Alterations were also observed in developing neuronal peripheral tissues of growth-retarded fetuses regarding axons and Schwann cells, which express S100B protein. In our study the loss of the correlation between gestational age and S100B protein in growth-retarded fetuses supports the hypothesis of an alteration in the normal process of development and maturation of the brain in this condition.

On the other hand, we cannot exclude the possibility that increased S100B protein concentration in peripheral blood of growth-retarded fetuses with decreased cerebral vessel resistance may derive from an increased leaking of the protein. Redistribution of fetal circulation is related to hypoxemia-mediated excessive release of vasoactive agents, which, in turn, may alter the permeability of the blood–brain barrier, accounting for increased S100B protein transfer from the tissue to the systemic circulation.

**Conclusion:**

This study shows that circulating S100B protein is increased in growth-retarded fetuses and correlates with fetal FVWs. Although the appearance in the blood of preterm infants with neurologic sequelae of fetal hypoxia markers such as nucleated erythrocytes and uric acid has been already reported, the release in the systemic circulation of brain constituents such as S100B protein, which is a direct indicator of active cell damage in the nervous system, may be a valuable biochemical marker to assess at birth fetal brain distress accompanying IUGR. The mechanism that gives rise to increased S100B protein and its relevance in the monitoring of IUGR fetuses, however, remains to be established.

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**References:**

38. Alex J Pediatr, 19(1), Jan 2005