

S100 beta protein is increased in fetuses with neural tube defect

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1. ABSTRACT

We investigated the levels of S100 beta protein (S100B) in the serum of fetuses with neural tube defects (NTD), and their mother. Samples from 20 fetuses with NTD and 30 controls at the same gestational age, and their mothers, were studied. S100B protein levels were determined using Lia-mat Sangtec kit. S100B concentrations were significantly higher in NTD fetuses (median 2.71 microg/L) than in control subjects (median 0.98 microg/L). Increased S100B levels were also found in mothers carrying fetuses with NTD compared to control and uncomplicated pregnancies. This study indicates that NTD is associated with increased serum concentration of S100B in fetuses and mothers. Moreover, it gives information on S100B levels in the fetal circulation in early-mid gestation.

2. INTRODUCTION

The S100 family of calcium binding proteins, first isolated in 1965 by Moore in a subcellular fraction from bovine brain, contains approximately 16 members each of which exhibits a unique pattern of tissue/cell type specific expression (1). Although the distribution of these proteins is not restricted to the nervous system, the implication of several members of this family in nervous system development, function, and disease has sparked new interest in these proteins. S100 beta protein (S100B) is one of the original two members of this family, it is an acidic calcium binding protein with a molecular weight of 21kD, present extracellularly, intracellularly, and in the cytosol; its half-life is of about 2 hours, and it is mainly eliminated by the kidney (2). S100B is present in central nervous system where it is mainly concentrated in the glial

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cells, astrocytes, Schwann cells and neurons. It regulates several cellular functions (cell-cell communication, cell growth, cell structure, energy metabolism, contraction and intracellular signal transduction) and it is expressed at elevated concentrations in brain damage (3). In this regard, S100B has been related with neurobehavioural abnormalities and microcephaly due to *in utero* cocaine exposure (4), and the appearance of S100B immunoreactivity cell was abnormal in anencephalic fetuses (5). Furthermore, S100B concentration in blood and in cerebrospinal fluid is increased in brain damage in adults and in infants (6,7) and the elevation of S100B has also been noted in chronic neurological conditions such as Down syndrome, dementia and schizophrenia. Previous studies have demonstrated age-related changes in S100B tissue expression and its distribution in the central nervous system in mammals (8,9), suggesting different roles for this protein in distinct brain regions during development. S100B protein is detectable in the umbilical cord blood of preterm and term fetuses and we observed a negative correlation between gestational age and serum S100B in late gestation in healthy fetuses (10) as demonstrated between age and circulating S100B in normal children (11). In previous studies we have shown that S100B is detectable in maternal circulation and it is increased in association with fetal growth impairment (12).

Neural tube defects (NTDs) are among the most common human congenital malformations. NTDs include all congenital anomalies that involve a failure of the neural tube to close during the fourth week of human embryogenesis, with defects occurring at any point along the formation of the spinal cord. NTDs formally comprise spina bifida (myelodysplasia or myelomeningocele), anencephaly, encephalocele, craniorachischisis, and iniencephaly. Spina bifida and anencephaly, the most common forms of NTDs, occur in approximately 300 000 newborns worldwide (13). The prevalence of NTDs has declined considerably during the past three decades due to advances in the refined resolution of ultrasonography for *in utero* fetal examination, termination of affected pregnancies, and folic acid supplements being widely consumed by women in the reproductive age group. NTDs etiology is quite complex and multifactorial, involving both environmental and genetic components. These defects vary in their severity depending on the type and the level of the lesion (14,15). S100B has been studied in fetal malformations (16,17) and increased S100B protein levels in plasma have been found in infants developing intraventricular hemorrhage and in those with chronic asphyxia, suggesting a role for this peptide as brain injury marker during fetal life (18,12). However, there is little information about the extracellular levels of this protein in NTDs subjects during brain development and growth.

The aim of this study was to investigate whether serum S100B protein concentration in NTDs fetuses is increased compared with other fetal diseases and whether NTDs are associated to elevated maternal S100B concentrations.

3. MATERIAL AND METHODS

3.1. Patients

Blood samples were collected from 20 fetuses with NTDs and their mothers who underwent pregnancies termination for the fetal malformation between 16 and 21 weeks of gestation. As control subjects 30 fetuses aborted at the same gestational age either spontaneously or induced for fetal or maternal indications, together with their mothers, were enrolled. Maternal blood samples were also collected from 20 pregnant women at the same gestational age (16-21 weeks of gestation) carrying healthy unaffected fetuses.

3.2. Samples collection

Blood samples were collected at the time of abortion from the umbilical cord vein. Maternal plasma was collected from cubital vein before the induction of abortion. Indications for pregnancy termination in control subjects (n= 20) included fetal malformations not affecting the brain and chromosomal abnormalities (not including trisomy 21). Fetuses aborted spontaneously between 16 and 21 weeks of gestation (n=10) underwent autopsy to exclude brain abnormalities. The protocol of the study was approved by the University Ethical Committee. Informed consent was obtained from all patients.

3.3. S100 measurement

Heparin-treated blood samples were immediately centrifuged at 900 g for 10 min and the supernatants stored at -70°C. The S100B concentration was measured in all samples by immunoluminometric assay (Lia-mat Sangtec 100, AB Sangtec Medical, Bromma, Sweden), according to the manufacturer's instructions. This assay is specific for the beta subunit of the S100 protein and measures the beta subunit by using three monoclonal antibodies (SMST 12, SMSK 25 and SMSK 28). Each measurement was performed in duplicate according to the manufacturer's recommendations and the averages were reported. According to the manufacturer's instructions, the sensitivity of the assay ($B_0 \pm 3SD$) was 0.02 microg/L, and the coefficient of variability was 5.5 % or lower within-assay and 10.1 % or lower inter-assay for concentrations ranging between 0.28 and 4.17 microg/L.

3.4. Statistical analysis

Statistical analyses were performed using Kruskal-Wallis 1-way ANOVA followed by Mann-Whitney 2-tailed test when indicated. Data are expressed as median and interquartile range (IQ 25/75). The Spearman correlation coefficient was used to evaluate correlation between fetal and maternal serum S100B.

4. RESULTS

As shown in figure 1, S100 B was significantly higher ($p < 0.01$) in NTDs fetuses (median= 2.71 microg/L; IQ= 2.03/3.74) compared to control subjects (median= 0.98 microg/L; IQ= 0.09/1.28). Pregnant women carrying NTDs fetuses had statistically significant higher ($p < 0.05$) levels of circulating S100B (median=0.36 microg/L; IQ

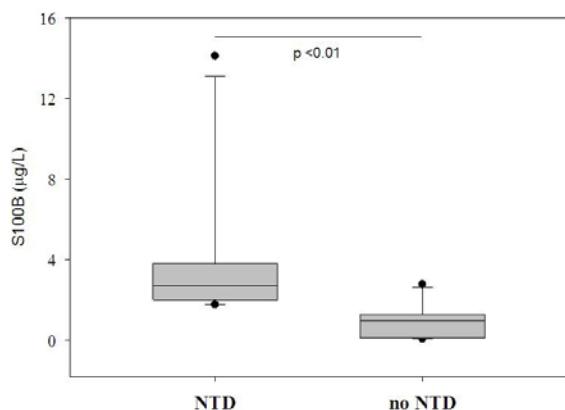


Figure 1. Concentrations of S100B in umbilical vein of fetuses with NTDs and without NTDs (no NTD). The lower and upper bars represent the 10th and 90th centiles, respectively, and the interquartile range is indicated by the box, the median value being the horizontal line in the box. S100B values were significantly higher ($p<0.01$) in NTDs fetuses.

0.16/2.69) compared with those with fetuses not affected by NTDs (median= 0.11 $\mu\text{g/L}$; IQ= 0.09/0.20) or with uncomplicated pregnancies (median= 0.22 $\mu\text{g/L}$; IQ= 0.12/0.21) at the same gestational age. However S100B concentration in fetal and maternal circulation were not correlated in either NTDs or control pregnancies.

5. DISCUSSION

This is the first study to show the concentration of S100B in fetuses with NTDs and their mothers. Moreover, this is the first study to show the levels of S100B in the fetal circulation in early-mid gestation. The origin of S100B in the umbilical cord veins could be in the fetal nervous system, which is known to contain the protein at the stages of development under examination. The pattern of accumulation of the protein in the human fetal development at these stages is essentially completed, excepting cortex, where the protein exhibits a late appearance (19). It may be noteworthy in this respect that the caudo-rostral pattern of accumulation of the protein has been related to the biochemical, morphological and electrophysiological maturation of the nervous system. We have previously reported that in the third trimester S100B in fetal circulation decreases with advancing gestation (10). In this study, however, we found that fetal S100B in early pregnancy is not higher than in late pregnancy, but rather slightly lower than in preterm fetuses. This is an intriguing finding which argues against a role of brain-barrier permeability in explaining fetal S100B negative correlation with gestational age in the third trimester of pregnancy and suggests a specific role of S100B as cytokine with neurotrophic effects in the time-related brain development processes.

We found that fetuses with NTDs had higher levels of S100B than fetuses with other abnormalities not affecting the nervous system. This finding is in accordance

with previous studies demonstrating increased S100B in amniotic fluid of anencephalic and NTDs fetuses (16,17) and suggests that the levels of S100B in fetuses with NTD could be considered a biological sign of cell injury of exencephalic brain. An experimental study in mice showed increased S100B in amniotic fluid of animals with spinal cord injury as a result of activation of the glial system (20). This mechanism may be responsible also for the increased levels of S100B in the circulation of fetuses with NTDs. Interestingly, Netto and coworkers failed to show an increase in S100B in infants with NTDs, although the levels did not decrease with age as demonstrated in normal children (21). We hypothesize that this over-expression of S100B in NTDs might occur very early during brain development and gives reason for the increased circulating concentrations. In pregnancy, S100B in maternal bloodstream has been suggested to be a potential marker for discriminate IUGR fetuses at higher risk for neurological complications such as intraventricular hemorrhage (12). Similarly, in this study we found that S100B is higher in the circulation of mothers carrying fetuses with NTDs compared to control pregnancies, although fetal and maternal concentrations were not correlated. Different factors might account for this discrepancy: placental perfusion, placental transfer, gestational age. The mechanism underlying S100B elevation in maternal circulation in all the conditions of elevated S100B in fetal circulation remains to be clarified.

We recognize that this study has some limitations, particularly because of the control group which should be constituted by healthy unaffected subjects. However, for obvious ethical reasons, this is not feasible when studies are performed on human fetuses in early pregnancy. To minimize the possible effect of fetal pathologies on S100B levels, we have selected fetuses with malformations not affecting nervous system such as kidney, limbs and abdominal wall, which are not associated with modification of circulating S100B. We measured S100B also in the fetuses with chromosomal abnormalities, however, since Down syndrome is associated with increased S100B levels (22), those affected by trisomy 21 have been excluded. Moreover, as further control, we measured S100B in the maternal circulation in uncomplicated pregnancies and we found no difference in the level of protein between these subjects and pregnant women with abnormal fetuses without NTDs used as controls.

In summary, we have shown that either maternal and fetal S100B is increased in NTDs fetuses in early-mid gestation. We speculate that this protein is a marker of brain injury, although the possible correlation with severity of neurological sequels has to be defined.

6. ACKNOWLEDGMENTS

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