

Obstetrics; Maternal-Fetal Medicine and Perinatology

Logical Basis of Cerebrospinal Fluid and Serum S-100B Protein Measurement in Pregnant Women to Detect any Possible Cerebral Damage

Nilüfer ÇELİK¹, Önder ÇELİK², Halis CANPOLAT³, Osman EVLİYAOĞLU¹, H. Muammer KARAKAŞ⁴

Malatya, Turkey

OBJECTIVE: The aim of the study was to consider logical basis of serum and cerebrospinal fluid (CSF) S100B measurement in pregnant females to detect any cerebral damage.

STUDY DESIGN: CSF and serum samples from 14 pregnant patients (age: 20-34 years) were obtained during spinal anesthetic procedure of cesarean section. The serum samples from 9 non-pregnant patients (22–36 years) without an organic brain disease were used as normative data. S-100B levels in serum and CSF samples were measured with electrochemiluminescence immunoassay method. The CSF and blood serum levels of pregnant and blood serum levels of pregnant and nonpregnant females were compared using Kruskal-Wallis and Mann-Whitney U-tests.

RESULTS: Serum S100B protein levels of pregnant females were significantly higher [0.66 ± 0.06 ng/ml] than those observed in [0.06 ± 0.00 ng/ml] nonpregnant females ($p < .0001$). There was a significant difference between the S100B protein levels of CSF and blood serum ($p < 0.05$) in pregnant females. However the correlation between these two levels was insignificant ($p = 0.473$). CSF S100B levels of pregnant subjects were significantly higher than the serum levels of pregnant and nonpregnant subjects ($p < 0.0001$).

DISCUSSION: These results prove that the maternal serum and CSF S100B protein levels are independently related to each other in pregnancy.

Key Words: S-100B, Pregnancy, Cerebrospinal fluid, Serum

Gynecol Obstet Rebrod Med;15:2 (81-84)

Introduction

The S100 is an acidic calcium-binding protein with a molecular weight of 21 kDa.¹ It is more predominant in astroglial cells and it acts on neuronal cells as a neurotrophic factor. Although the function of S100 is not fully known, it was shown to be involved in the regulation of cell growth.¹⁻³ Initially regarded as a central nervous system (CNS) specific protein⁴, it actually exists in diverse non-neural locations such as striated muscles, heart, kidney,⁵ melanocytes, adrenal glands, Leydig cells, adipocytes and trophoblastic cells.⁶⁻⁸ As it is predominantly found in the CNS, many authors have suggested that an increase in S100B CSF could be a potential marker of neural injury, and increase in blood could be a potential marker of blood brain barrier dysfunction.⁹

¹Beydağı State Hospital Clinical Biochemistry, ²Departments of Obstetrics and Gynecology, ³Anesthesiology and ⁴Radiology İnönü University School of Medicine, Malatya

Address of Correspondence: Nilüfer Çelik
Beydağı State Hospital, Clinical
Biochemistry, Malatya
nilufercelik35@hotmail.com

Submitted for Publication: 07.05.2009

Accepted for Publication: 15.06.2009

Accordingly, increased S100B levels in CSF and blood have encountered in several pathologic conditions, such as head trauma,¹⁰ stroke,¹¹ schizophrenia,¹² cerebral bleeding, ischemic stroke, brain tumours, meningitis and encephalitis.¹³⁻¹⁶

The studies on S100B levels in maternal serum, amniotic fluid and cord blood are very limited.¹⁷⁻²⁰ This protein has been shown to be higher in the amniotic fluid of monoamniotic twins than of singleton pregnancies.^{21,22} Increased S100B protein levels was also been found in amniotic fluid of fetuses with Down syndrome.²³ S100B protein levels in the cord blood were found to be higher in vaginal delivery than in cesarean section.²⁴ CSF S100B levels, on the other hand, have not been previously reported in pregnancy and its clinical significance in pregnancy therefore was indetermined. There may be hypothetical cerebral and BBB changes during this physiological event, and the determination of CSF and blood serum S100B levels may help us to disclose these changes indirectly. The purpose of this study, therefore, was to consider logical basis of serum S100B protein measurement in pregnant females to detect any possible cerebral damage. S100B protein in maternal CSF and blood serum were measured and the findings were evaluated with the ones obtained from the blood serum of non-pregnant females. This is also the first report in the literature comparing serum and CSF S100B protein levels in pregnancy.

Material and Method

Nine non-pregnant and fourteen pregnant women (thirty four or higher gestational weeks) were included in the study. Exclusion criteria for pregnant and non-pregnant women were as follows: Brain damage following head trauma, cerebral bleeding, ischemic stroke or known medical history that implies neurological conditions causing acute and chronic brain injury. Estimated gestational age was determined by using the date of the last menstrual period and it was also verified with ultrasonography. These pregnancies were terminated with cesarean section (C/S). In the course of the procedure a spinal anesthesia was performed. A 4-mL venous blood sample was taken by venipuncture before a 0.9% NaCl infusion. After the infusion, spinal anesthesia was performed in a seated position from the fourth to fifth lumbar space with a 25 gauge Quincke needle. Before the administration of local anesthetic to the subdural space, 2 mL of CSF was taken from the subjects. Subjects with hemorrhagic samples were excluded from the study. Blood samples were also taken from nonpregnant women by venipuncture. To determine the S100B protein level, CSF and blood samples were centrifuged on receipt and stored at -50 °C until assayed. S-100B levels in serum and CSF samples were measured with electrochemiluminescence immunoassay method on the Roche Elecsys 2010 immunoassay analyzer (Roche Elecsys 2010, Mannheim, Germany) using commercial S100B kit (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Results are given as means \pm SEM. The Statistical Package for Social Sciences (SPSS), version 13.0, was used for statistical analysis. Individual group parameters were assessed with one-sample Kolmogorov-Smirnov Z-test and were found to be abnormally distributed ($p < 0.05$). Statistical comparisons between groups were performed by nonparametric Kruskal-Wallis and Mann-Whitney U-test. Spearman's Rank Order Correlation coefficients were used to assess meaningful associations between the maternal serum and CSF S100B protein levels. For all comparisons, statistical significance was defined as $p < 0.05$.

Results

In pregnant women, there was a significant difference between CSF S100B [1.08 ± 0.14 ng/ml] and serum S100B [0.66 ± 0.06 ng/ml] protein levels ($p < 0.004$). Although statistically insignificant, a negative correlation was existed between maternal serum and maternal CSF S100B levels ($r = -0.209$, $p = 0.473$). Serum S100B protein levels of pregnant women were significantly higher [0.66 ± 0.06 ng/ml] than those observed in [0.06 ± 0.00 ng/ml] nonpregnant women ($p < 0.0001$). CSF-S100B protein levels in pregnant women were higher than serum S100B protein levels in nonpregnant subjects [1.08

± 0.14 ng/ml vs. 0.06 ± 0.00 ng/ml; $p < .0001$] (Figure 1).

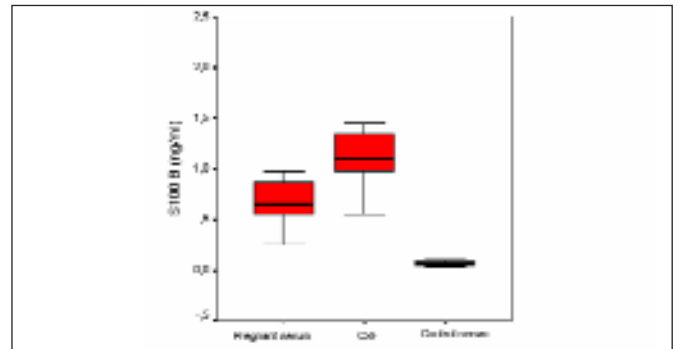


Figure 1: Maternal serum and CSF S100B protein levels of fourteen pregnant women and serum S100B protein levels of nine nonpregnant women

Discussion

This study is the first investigation in the literature that was designed to assess CSF S100B in pregnancy. In that group its mean level in CSF was found to be 1.08 ng/ml. CSF S100B levels were not compared between pregnant and nonpregnant controls due to the nature of the latter. These were all non-pregnant females that prevented the use of an invasive procedure (i.e. CSF tapping). However, the data may be compared with the results of previous studies on normal subjects.^{9, 16, 25} According to these studies the mean concentration in CSF samples obtained from individuals with no evidence of neurological disease varies between 0.20 and 1.5 ng/ml.²⁵⁻²⁷ Persson et al.⁹ quote a wider range that encompasses 1.0 to 6.8 ng/ml whereas Noppe et al.¹⁶ reported a value less than 0.8 ng/ml. Our findings were similar to the ones listed above except the latter value which is slightly lower.

The second step of the study was conducted to reveal a possible difference between pregnant and nonpregnant women regarding serum S100B protein levels. Considering the similarity of CSF S100B levels in both groups studied, any observed differences in serum levels should originate from extraneural sources. This protein was found to have a serum level of 0.66 ng/ml in pregnant women, which was significantly higher than those observed in nonpregnant women (0.06 ng/ml). Although the level in pregnant was much higher than the one that is found by Abraha et al.²⁰ (0.11 ng/ml), the advanced gestational range of our subjects (36-40 wk vs. 11-38 wk) explains the difference.

In spite of the high S100B levels in maternal serum the origin of this increase is not clear. Being a neural marker, its increase in maternal serum may either show a subtle brain barrier dysfunction which may facilitate the leakage of the protein to the blood circulation or may be the result of secretion from extracerebral sources.²⁸ Due to the insignificant correlation between CSF and serum S100B protein levels, it is un-

likely that CSF-S100B protein may be responsible for the increase in serum S100B concentrations.

Although there is not another comparable study that documents the relationships between maternal CSF and serum levels of S100B, there are some studies that have documented an inverse relationship similar to one stated above. In our previous study we had demonstrated an inverse relationship between CSF and serum leptin levels in normotensive pregnant subjects.²⁹ We also found that independent regulation of nitric oxide in the CSF and serum compartments of normotensive and preeclamptic women.³⁰ The current study complements the previous studies and supports our hypothesis that the cerebrovascular and blood brain barrier alterations in uncomplicated pregnancy are not directly linked to the protein escape from CSF into the maternal circulation. Our hypothesis on the lack of blood brain barrier dysfunction, is further supported by the findings of Abraha et al³¹ who investigated subjects with neurological damage. Although the subjects of that study had high S100B concentrations, only a small fraction of the protein was found to be released to the serum.

To explain the significant increase of S100B in the serum, diverse non-neural locations as stated in the introduction should be considered. In pregnant subjects, the causative factors may likely be the ones related to gestation such as amnion, villous and intermediate trophoblasts, decidual cells of fetal membranes and in endothelial cells of umbilical vessels at all gestational ages.⁶⁻⁸ Several studies that support our findings were performed during previous years.^{18,19} Marinoni et al.⁷ reported an increase in S100B protein concentration in normal placenta with advancing gestation. Gazzolo et al.³² observed a positive correlation between S100B level and gestational age in amniotic fluid. Another source of increased serum S100B protein levels may be adipose tissue. Michetti et al⁸ reported that adipocytes contain S100B protein in concentrations comparable to the nervous tissue. Results of the recent prospective study indicate that subcutaneous fat begins to accumulate around 6 weeks after conception and continues to increase through 35 weeks of pregnancy.³³

Finally, we suggest that pregnancy related physiologic changes may alter serum S100B protein levels but this effect are not accompanied by changes in the S100B content in CSF. Taken together, our own findings and data from the literature suggest the possibility of a fetal-placental or adipocyte origin for at least a part of S100B protein detected in the serum. CSF-S100B protein appears not to influence the levels of serum S100B protein. Therefore, S100B protein should not be used to predict any neural damage in pregnancy as extracerebral sources largely affect its levels in the latter. In pregnancy, prominent contribution of placenta and fetal sources may obscure any slight deviation that would be caused by cerebral structures.

Gebe Kadınlarda Beyin Omurilik Sıvısı ve Serum S-100B Protein Ölçümünün Serebral Hasarı Saptamadaki Rolü

AMAÇ: Bu çalışma serum ve beyin omurilik sıvısı (BOS) S100B protein düzey ölçümlerinin gebelerde serebral hasarı saptamadaki rolünü araştırmak için planlandı.

GEREÇ VE YÖNTEM: Yaşları 20-34 arasında değişen 14 gebe hastadan sezaryen operasyonu için yapılan spinal anestezi esnasında serum ve BOS örnekleri alındı. Gebe olmayan ve organik bir beyin hastalığı bulunmayan 9 sağlıklı kadından alınan serum örnekleri ise kontrol grubu olarak belirlendi. Serum ve BOS S100B protein düzeyleri electrochemiluminescence yöntemiyle çalışıldı. Kontrol grubu ve gebelerin serum ve BOS S100B düzeyleri Kruskal-Wallis ve Mann-Whitney U-test kullanılarak karşılaştırıldı.

BULGULAR: Gebe kadınların serum S100B protein düzeyleri [0.66 ± 0.06 ng/ml] gebe olmayanlardan [0.06 ± 0.00 ng/ml] anlamlı olarak daha yüksek saptandı ($p<0.0001$). Gebe kadınların BOS S100B protein düzeyleri serum S100B protein düzeylerinden anlamlı olarak yüksek bulundu ($p<0.05$). Ancak iki düzey arasındaki korelasyon anlamlı değildi ($p=0.473$). Gebelerde BOS S100B protein düzeyleri gebe olmayanların serum S100B protein düzeylerinden anlamlı olarak yüksek saptandı ($p<0.0001$).

SONUÇ: Gebelerde serum ve BOS S100B protein düzeyleri birbirinden bağımsız olarak regüle edilir.

Anahtar Kelimeler: S100B protein, Gebelik, Beyin omurilik sıvısı, Serum

References

1. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int J Biochem Cell Biol.* 2001;33:637-68.
2. Marks A, Allore R. S100 protein and Down syndrome. *Bioessays.* 1990;12:381-3.
3. Endo T, Tanaka T, Isobe T, Kasai H, Okuyama T, Hidaka H. Calcium-dependent affinity chromatography of S-100 and calmodulin on calmodulin antagonist-coupled Sepharose. *J Biol Chem.* 1981;256:12485-9.
4. Rickmann M, Wolff JR. S100 protein expression in subpopulations of neurons of rat brain. *Neuroscience.* 1995;67:977-91.
5. Isobe T, Takahashi K, Okuyama T. S100a0 (alpha alpha) protein is present in neurons of the central and peripheral nervous system. *J Neurochem.* 1984;43:1494-6.
6. Haimoto H, Hosoda S, Kato K. Differential distribution of immunoreactive S100-alpha and S100-beta proteins in normal nonnervous human tissues. *Lab Invest.* 1987;57:489-98.
7. Marinoni E, Di Iorio R, Gazzolo D, Lucchini C, Michetti F, Corvino V, Cosmi EV. Ontogenetic localization and dis-

- tribution of S-100beta protein in human placental tissues. *Obstet Gynecol.* 2002;99:1093-9.
8. Michetti F, Dell'Anna E, Tiberio G, Cocchia D. Immunochemical and immunocytochemical study of S-100 protein in rat adipocytes. *Brain Res.* 1983;262:352-6.
 9. Persson L, Hardemark HG, Gustafsson J, Rundstrom G, Mendel-Hartvig I, Esscher T, Pahlman S. S-100 protein and neuron-specific enolase in cerebrospinal fluid and serum: markers of cell damage in human central nervous system. *Stroke.* 1987;18:911-8.
 10. Ingebrigtsen T, Waterloo K, Jacobsen EA, Langbakk B, Romner B. Traumatic brain damage in minor head injury: relation of serum S-100 protein measurements to magnetic resonance imaging and neurobehavioral outcome. *Neurosurgery.* 1999;45:468-75; discussion 475-6.
 11. Wunderlich MT, Ebert AD, Kratz T, Goertler M, Jost S, Herrmann M. Early neurobehavioral outcome after stroke is related to release of neurobiochemical markers of brain damage. *Stroke.* 1999;30:1190-5.
 12. Lara DR, Gama CS, Belmonte-de-Abreu P, Portela LV, Goncalves CA, Fonseca M, Hauck S, Souza DO. Increased serum S100B protein in schizophrenia: a study in medication-free patients. *J Psychiatr Res.* 2001;35:11-4.
 13. Michetti F, Massaro A, Russo G, Rigon G. The S-100 antigen in cerebrospinal fluid as a possible index of cell injury in the nervous system. *J Neurol Sci.* 1980;44:259-63.
 14. Sindic CJ, Chalon MP, Cambiaso CL, Laterre EC, Masson PL. Assessment of damage to the central nervous system by determination of S-100 protein in the cerebrospinal fluid. *J Neurol Neurosurg Psychiatry.* 1982;45:1130-5.
 15. Mokuno K, Kato K, Kawai K, Matsuoka Y, Yanagi T, Sobue I. Neuron-specific enolase and S-100 protein levels in cerebrospinal fluid of patients with various neurological diseases. *J Neurol Sci.* 1983;60:443-51.
 16. Noppe M, Crols R, Andries D, Lowenthal A. Determination in human cerebrospinal fluid of glial fibrillary acidic protein, S-100 and myelin basic protein as indices of non-specific or specific central nervous tissue pathology. *Clin Chim Acta.* 1986;155:143-50.
 17. Schmidt AP, Tort AB, Amaral OB, Schmidt AP, Walz R, Vettorazzi-Stuckzynski J, Martins-Costa SH, Ramos JG, Souza DO, Portela LV. Serum S100B in pregnancy-related hypertensive disorders: a case-control study. *Clin Chem.* 2004;50:435-8.
 18. Gazzolo D, Marinoni E, di Iorio R, Lituania M, Bruschetini PL, Michetti F. Circulating S100beta protein is increased in intrauterine growth-retarded fetuses. *Pediatr Res.* 2002;51:215-9.
 19. Gazzolo D, Vinesi P, Marinoni E, Di Iorio R, Marras M, Lituania M, Bruschetini P, Michetti F. S100B protein concentrations in cord blood: correlations with gestational age in term and preterm deliveries. *Clin Chem.* 2000;46:998-1000.
 20. Abraha HD, Noble PL, Nicolaidis KH, Sherwood RA. Maternal serum S100 protein in normal and Down syndrome pregnancies. *Prenat Diagn.* 1999;19:334-6.
 21. Michetti F, Gazzolo D. S100B testing in pregnancy. *Clin Chim Acta.* 2003;335:1-7.
 22. Heizmann CW. Ca²⁺-binding S100 proteins in the central nervous system. *Neurochem Res.* 1999;24:1097-100.
 23. Gazzolo D, Bruschetini M, Corvino V, Lituania M, Sarli R, Bruschetini P, Michetti F. Amniotic fluid levels of S100B protein in normal and trisomy-21 fetuses. *Clin Chim Acta.* 2003;330:131-3.
 24. Wirds JW, Duyn AE, Geraerts SD, Preijer E, Van Diemen-Steenvoorde JA, Van Leeuwen JH, Haas FJ, Gerritsen WB, De Boer A, Leusink JA. S100 protein content of umbilical cord blood in healthy newborns in relation to mode of delivery. *Arch Dis Child Fetal Neonatal Ed.* 2003;88:F67-9.
 25. Green AJ, Keir G, Thompson EJ. A specific and sensitive ELISA for measuring S-100b in cerebrospinal fluid. *J Immunol Methods.* 1997;205:35-41.
 26. van Engelen BG, Lamers KJ, Gabreels FJ, Wevers RA, van Geel WJ, Borm GF. Age-related changes of neuron-specific enolase, S-100 protein, and myelin basic protein concentrations in cerebrospinal fluid. *Clin Chem.* 1992;38:813-6.
 27. Reiber H. Dynamics of brain-derived proteins in cerebrospinal fluid. *Clin Chim Acta.* 2001;310:173-86.
 28. Missler U, Orłowski N, Notzold A, Dibbelt L, Steinmeier E, Wiesmann M. Early elevation of S-100B protein in blood after cardiac surgery is not a predictor of ischemic cerebral injury. *Clin Chim Acta.* 2002;321:29-33.
 29. Celik O, Hascalik S, Ozerol E, Hascalik M, Yologlu S. Cerebrospinal fluid leptin levels in preeclampsia: relation to maternal serum leptin levels. *Acta Obstet Gynecol Scand.* 2004;83:519-23.
 30. Celik O, Hascalik S, Turkoz Y, Hascalik M, Gokdeniz R. Cerebrospinal fluid nitric oxide level changes in preeclampsia. *Eur J Obstet Gynecol Reprod Biol.* 2003;111:141-5.
 31. Abraha HD, Butterworth RJ, Bath PM, Wassif WS, Garthwaite J, Sherwood RA. Serum S-100 protein, relationship to clinical outcome in acute stroke. *Ann Clin Biochem.* 1997;34:366-70.
 32. Gazzolo D, Bruschetini M, Corvino V, Oliva R, Sarli R, Lituania M, Bruschetini P, Michetti F. S100b protein concentrations in amniotic fluid correlate with gestational age and with cerebral ultrasound scanning results in healthy fetuses. *Clin Chem.* 2001;47:954-6.
 33. Sidebottom AC, Brown JE, Jacobs DR Jr. Pregnancy-related changes in body fat. *Eur J Obstet Gynecol Reprod Biol.* 2001;94:216-23.