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S100B Protein in Serum as a Prognostic Marker for Brain Injury in Term Newborn Infants with Hypoxic Ischemic Encephalopathy - New Strategy for Early Brain Damage

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Abstract

Background: The aim was to investigate whether S100 in serum is a prognostic marker of cerebral injury in term newborn infants with hypoxic ischemic encephalopathy (HIE) after perinatal asphyxia.

Material and Methods: All risk neonates with severe asphyxia, admitted to the neonatal and Pediatric Intensive Care Unit at the University Pediatric Hospital in Skopje-Macedonia within 24h of injury were eligible for inclusion in the study. One serum blood sample was obtained from each patient at the 24h post-injury time-point, than day 3 and day 7. S100B levels were measured using ECLIA method (Electro-Chemil-Luminiscence Immuno Assay-Elecsys 2010-Roche Diagnostic).

Results: One hundred and nineteen neonates were recruited. The avarage serum S100B levels for the control group (N=48) was 0.12 microgL(-1) (cut-off point). S100B levels were significantly higher in asphyxiated term neonates N=29; M= 0.64. Infants with moderate and severe HIE had significantly higher S100 levels on postnatal day 1 ($p = 0.031$) and day 2 ($p = 0.008$) than infants with mild or no HIE. Increased S100 levels were significantly inversely correlated with perinatal pH in the infants and associated with abnormal CTG at admission to the labor ward.

Conclusion: Early determination of serum S100 may reflect the extent of brain damage in infants with HIE after asphyxia.

Introduction

Hypoxic-ischemic encephalopathy (HIE) is a condition with great impact on the body of the new born infant, being the result of perinatal asphyxia, this encephalopathy compromises several organs, in addition to causing possible sequelae such as cerebral palsy, epilepsy and mental retardation [1, 2]. Its clinical signs are the progressive involvement of neurological functions, including breathing maintenance or onset, tonus, reflexes and strength, change in consciousness and frequent seizures. According to the literature, the incidence of HIE varies between 0.1-0.4% of births, with diagnosis being essentially clinical [2, 3]. The central nervous system (CNS) involvement varies with gestational age, nature of the damage and type of treatment. Premature infants are known to have more injuries in deeper, periventricular are as, while in term newborn infants

injuries are mostly located in the cortical- subcortical area [2, 4]. Depending on a complex biochemical cascade, different forms of neuronal death may occur. Several changes in anaerobic metabolism occur in case of decreased blood supply to the brain, such as glycolysis, increased concentrations of inorganic phosphates and lactate [4, 5]. Currently, for the diagnosis of HIE, in addition to medical history and proper neurological examination, metabolic parameters are becoming increasingly important. Diagnosis methods such as EEG, CT- scans, MRI and somatosensory potential are useful for pro gnosis, but not in the first 24 hours, while spectroscopy resonance has cost limitations1 [6-8].

A large number of molecules are assigned a role as markers of neurological injury in the presence of neonatal asphyxia. Glutamate, aspartate, lactate, ammonia, creatinine, specific kinase, NSE *(neuron specific enolase)* and other substances have already been studied in peripheral blood, umbilical cord blood, amniotic fluid and cerebrospinal fluid (CSF). Studies that measured the concentration of S100B, the calcium-binding protein that prevails in astrocytes, in the CSF and blood, have shown a direct relationship with brain injury [13, 14].

Significant contributions in the area of perinatology, such as in the administration of nitric oxide, brain hemorrhage studies and analysis of amniotic fluid in twins have shown that S100B protein is useful as a brain injury marker [12, 16, 17]. Additionally, its prognostic role was shown when it was associated with HIE in term newborn infants and a relation with moderate and severe stages could be found [12, 18].

S100B in vitro has a neurotrophic activity for neuronal cells during the neuronal maturation and glial cell proliferation. S100B decreases cell death and the loss of mitochondrial function resulting from glucose deprivation. With its neurotrophic and gliotrophic actions, S100B probably plays important roles in normal CNS development and recovery after injury. In contrast to the stated effects of nanomolar levels of S100B, micromolar levels of extracellular S100B may have deleterious effects. At these concentrations, extracellular S100B in vitro stimulates the expression of proinflammatory cytokines and induces apoptosis. S100B exerts its neurotoxic effects in vitro by inducing apoptosis in neurons.

Recent observations show that micromolar concentrations of S100B produce apoptotic death by interacting with the Receptor for Advanced Glycation End Products (RAGE), causing elevation in reactive oxygen species, cytochrome C release and activation of the caspase cascade. S100B might contribute to neuropathological changes in the course of neurodegeneration and/or brain inflammatory diseases by the activation of microglia as well. When a metabolic injury occurs, such as the deprivation of oxygen, serum and glucose, the early process during the glial response is the secretion of S100B. The high concentrations of S100B cause neuronal death through nitric oxide release from astrocytes. The biological half-life of S100B approximates 30 minutes. This implies that any persistent elevation of its serum levels reflects continuous release from affected tissues. Besides peripheral blood, S100B can be found in cord blood, urine, cerebrospinal fluid (CSF), amniotic fluid, and with markedly higher concentrations than these, in milk. The S100B content in serum is lower than that in CSF. Many extracerebral sources contribute to the serum S100B content. Immunoassays and mRNA quantification have characterized other cells as S100B-expressing cells, particularly adipocytes, chondrocytes, lymphocytes, bone marrow cells, and melanoma cells. These data explain why controversy has arisen in recent years as to the origin of serum S100B and the involvement of brain damage, or not, in this release.

Table 1: Cell-type specific S100B- and GFAP-immunostaining pattern.

Cell type	S100B	GFAP
Astrocytes	++, cytoplasm/nuclei	++, cytoplasm and processes
Oligodendrocytes	++, cytoplasm/nuclei	0
Neurones	very few +, cytoplasm/nuclei	0
Ependyma	++, cytoplasm/nuclei	+, cytoplasm
Choroid plexus epithelium	++, cytoplasm/nuclei	0
Lymphocytes	++, cytoplasm/nuclei	0

Annotation: 0 immunonegative, + faint immunostaining, ++ strong immunostaining.

The differential S100B- and GFAP-immunostaining pattern is summarized in Table 1 and Table 2.

S100B is not only implicated in the regulation of intracellular processes, but, it is also a secretory protein and exhibits cytokine-like activities, which mediate the interactions among glial cells and between glial cells and

Table 2: Quantification of S100B immunopositive cells with astrocytic versus oligodendrocytic morphology.

Brain region	Oligodendrocytic S100B+ [cells/mm^3]	Astrocytic S100B+ [cells/mm^3]	Oligodendrocytic S100B+ cells [mean % of all S100B+ glial cells (min % – max %)]
DLPF cortex	1.247 ± 672	5.066 ± 2.674	20 (14 – 30)
DLPF white matter	14.423 ± 7.061	4.462 ± 2.376	75 (57 – 85)
Temporal cortex	1.207 ± 860	7.457 ± 3.964	14 (7 – 35)
Temporal white matter	11.229 ± 5.085	3.919 ± 2.310	73 (59 – 87)
Parietal white matter	9.279 ± 4.074	2.346 ± 1.357	79 (62 – 89)
Corpus callosum	10.038 ± 4.533	674 ± 338	93 (86 – 97)

Annotation: Values are given as mean ± S.D.; *DLPF,* dorsolateral prefrontal.

neurones. Interaction of S100B with the receptor for advanced glycation end products (RAGE), a multiligand receptor that has been shown to transduce inflammatory stimuli and effects of several neurotrophic and neurotoxic factors . Secretion of S100B from astrocytes is stimulated under metabolic stress (oxygen, serum and glucose deprivation) and is suppressed by glutamate [S100B acts in a dose-dependent manner: Nanomolar levels stimulate neurite growth and promote neurone survival. Micromolar levels result in opposite effects and can even induce neuronal apoptosis, leading to the induction of pro-inflammatory cytokines such as interleukin1β (IL-1β) or tumour necrosis factor α (TNF-α), and inflammatory stress-related enzymes such as inducible nitric oxide synthase (iNOS) [12, 13].

Birth asphyxia remains a considerable problem in perinatal medicine with an incidence around 0.6-0.8% of births [30-32]. About half of these infants develop hypoxic ischemic encephalopathy (HIE) [32-34]. Those with moderate and severe HIE are at high risk of developing cerebral palsy (CP) [35-38].Prognostic assessment of brain injury after perinatal asphyxia will become essential for proper selection concerning early cerebroprotective treatment, which might be a likely option in the near future [39, 40]. Several biochemical markers in cerebrospinal fluid (CSF) have been associated with cerebral complications in infants after perinatal asphyxia [41-43] and recently also S100 in CSF [44, 45]. A prognostic marker in serum would be of great value [46, 47]. The astroglial protein S100 is an established biochemical marker for CNS injury in the adult, in whom increased levels of S100 in CSF have been reported after cerebral insults [48, 49]. S100 in blood has been shown to be a marker of brain damage in adult stroke [50, 51] and a potential marker for cerebral events after cardiac arrest [52]. There are a few studies on S100 in serum of infants. S100 in serum has been shown to be a possible marker of postperfusion cerebral injury after pediatric cardiac operations [53]. In preterm infants with intraventricular hemorrhage concentrations of S100 in blood were elevated, before a radiologic assessment of hemorrhage could be performed [54]. Circulating S100 protein was increased in IUGR fetuses and correlated with cerebral hemodynamics, suggesting that it may represent an index of cerebral cell damage in the perinatal period [54]. Elevated S100 in serum measured during the first 24 h after asphyxia has been shown to be associated with HIE in term infants [55]. There have been recently described reference values of S100 in cord blood of newborn term infants with uncomplicated delivery [56] and in a pilot study there have been also found the increased S100 to correlate with the degree of HIE [57].

The aim of this study was further to investigate whether increased S100 levels in serum are correlated with the grade of HIE after perinatal asphyxia, mechanical ventilation in some severe cases of the asphyxiated infants and more specifically whether increased S100 predicts the cerebral injury and subsequent cerebral palsy.

Methods and Patients

All risk neonates with severe asphyxia, admitted to the Neonatal and Pediatric Intensive Care Unit at the University Pediatric Hospital in Skopje-Macedonia within 24h of injury were eligible for inclusion in the study from january 2010-2011 (N=29). One serum blood sample was obtained from each patient at the 24h post-injury time-point,than at 3rd and 7th day after the admission. S100B levels were measured using ECLIA method (Electro-Chemil-Luminiscence Immuno Assay-Elecsys 2010-Roche Diagnostic at the Biochemestry Clinic**.**

Study group

Twenty nine term newborn infants with birth asphyxia born between January 2010-2011 and treated at the Neonatal Intensive Care Unit (NICU), University Children's Hospital-Skopje, were prospectively included in the study. The diagnosis of asphyxia was made on clinical signs during the first hours of life, together with acid-base status.

The following inclusion criteria were used (all necessary):

· 1/Term newborn (≥ 36 completed gestational weeks), 2/Apgar score < 7 at 5 min or other clinical signs of perinatal asphyxia). Clinical signs of asphyxia necessitating transfer to neonatal intensive care unit. Some of them needed mechanical ventilation.

· All infants were neurologically examined daily during the first week of life especially had ultrasound at the 3rd and 7th day and classified according to the degree of HIE into mild, moderate or severe HIE. All infants had neurologic follow-up examinations to the age of at least 6 month. Assessment of outcome was done according to items from Amiel-Tison Infants were classified as having impairment or no signs of impairment at 6month-one year. CP was identified and classified according to the criteria of Hagberg.

Control group

Serum samples from the control group of 48 term newborn infants, gestational age, median (range) 40 weeks, were collected before the study started. The control infants, all with Apgar score ≥ 9 at 1.5 and 10 min.

Statistical analysis

Nonparametric tests were used. Correlations were made by Spearman's test, and for comparison of continuous variables betweeen groups, Mann-Whitney test was used. When using statistics for categorical data (Pearson χ^2 or Fisher's exact test) we assumed S100 < 12 μg/L as low and S100 ≥12 μg/L as high values. A p-value < 0.05 was regarded as significant.

Results

Because the data in the first two measurements time-points doesn't stand up to the parameters for repeated measures ANOVA we substitute with the Friedman test.

Table 3: The Friedman test values.

Measurement	N	Mean (AM)	Standard deviation (SD)	Min	Max	Mean rank
First day	29	0.642	0.352	0.100	1.350	1.40
4th day	29	0.680	0.414	0.090	1.900	2.07
7th day	29	1.078	1.001	0.020	4.300	2.53

The Friedman test value χ^2 (2; N= 29) is 19.32, (p<0.001) in all three measurements are statistically significant.

The Wilcoxon test value are statistically significant in the measurements of S100B protein between the 1st and 4th day (on the level 0.05 where the value is higher on the 4th day); between the 7th and 4th day (on the level 0.005 where the value is higher on the 7th day); between the 7th and 1st day (on the level 0.007 where the value is higher on the 7th day).

Table4: Wilcoxon Signed Ranks Test.

Value of Wilcoxon test	4th day-1st day	7th day-4th day	7th day-1st day
Z	-1.983 (a)	-2.881 (a)	-3.179 (a)
Asymp. Sig. (2-tailed)	0.047	0.004	0.001

a, Based on negative ranks; b, Wilcoxon Signed Ranks Test.

Examination of the coefficients for the linear combinations distinguishing groups on/off mechanical ventilation indicated that day 4 and day 7 contributed most to distinguishing the groups. In particular, both day 4 (-0.313) and day 7 (-1.073) contributed significantly toward discriminating between the group not on mechanical ventilation from the other group ($p < 0.05$ and $p < 0.005$, respectively). On the other hand, day 1 did not contribute significantly to distinguishing between the serum S100B levels in both groups.

Table 5: Means and Standard Deviations for serum S100B values at each day of measurement as a function of mechanical ventilation in the group of preterm neonates.

Mechanical Ventilation		N	Mean	SD
Day 1	Yes	14	0.168	0.254
	No	16	0.197	0.253
Day 4	Yes	14	0.416	0.338
	No	16	0.354	0.365
Day 7	Yes	14	0.614	0.394
	No	16	0.369	0.363

Table 6: Means and Standard Deviations for serum S100B values at each day of measurement as a function of mechanical ventilation in the group of term neonates with asphyxia.

Mechanical ventilation		N	Mean	SD
Day 1	Yes	16	0.564	0.333
	No	13	0.738	0.365
Day 4	Yes	16	0.540	0.323
	No	13	0.853	0.459
Day 7	Yes	16	0.597	0.516
	No	13	1.670	1.147

Follow up univariate ANOVAs (see Table 2) indicated that the serum S100B levels at both day 4 and day 7 were significantly different for the group on and the group not on mechanical ventilation, F (1) = 4.63, p = 0.040 and F (1) = 11.26, p = 0.002, respectively.

For prediction of neonatal outcome measured as moderate or severe HIE, the sensitivity of S100 > 12

Table 7: Tests of Between-Subjects Effects.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Day 1	0.216(a)	1	0.216	1.790	0.192	0.062
	Day 4	0.703(b)	1	0.703	4.633	0.040	0.146
	Day 7	8.253(c)	1	8.253	11.262	0.002	0.294
Intercept	Day 1	12.159	1	12.159	100.623	0.000	0.788
	Day 4	13.911	1	13.911	91.707	0.000	0.773
	Day 7	36.871	1	36.871	50.314	0.000	0.651
Mechanic Supprot	Day 1	0.216	1	0.216	1.790	0.192	0.062
	Day 4	0.703	1	0.703	4.633	0.040	0.146
	Day 7	8.253	1	8.253	11.262	0.002	0.294
Error	Day 1	3.263	27	0.121			
	Day 4	4.096	27	0.152			
	Day 7	19.786	27	0.733			
Total	Day 1	15.433	29				
	Day 4	18.213	29				
	Day 7	61.750	29				
Corrected Total	Day 1	3.479	28				
	Day 4	4.798	28				
	Day 7	28.039	28				

a) R Squared = 0.062 (Adjusted R Squared = 0.027); b) R Squared =0.146 (Adjusted R Squared = 0.115); c) R Squared = 0.294 (Adjusted R Squared = 0.268).

Table 8: Statistical descriptors for the S100B measurements in the group of term neonates with asphyxia.

Time Intervals	N	Mean	Standard Deviation	Min	Max	Mean Rank
24h (1st day)	29	0.642	0.352	0.100	1.350	1.40
4th day	29	0.680	0.414	0.090	1.900	2.07
7th day	29	1.078	1.001	0.020	4.300	2.53

μg/L was 50%, specificity 85%, positive predictive value (PPV) 82% and negative predictive value (NPV) 55%. For prediction of death or development of CP by 1 1/2 year, the sensitivity of S100 >12 μg/L was 58%, specificity 75%, PPV 50% and NPV 80% describes the prediction for HIE and outcome of S100 > 12 μL/g.

Discussion

The Wilcoxon test value are statistically significant in the measurements of S100B protein between the 1st and 4th day (on the level 0.05 where the value is higher on the 4th day); between the 7th and 4th day (on the level 0,005 where the value is higher on the 7th day); between the 7th and 1st day (on the level 0.007 where the value is higher on the 7th day). So, we can conclude that S100B protein is higher at asphyxiated term neonates with high risk for hypoxic ischemic encephalopathy, and S100B protein is a excellent marker for acute brain injury. The Mann-Whitney U test were not found the statistically significant difference in the value of S100B protein between the examination group with mechanical ventilation and the other group without that treatment in the first measuring at the risk group of asphyxiated neonates. Mechanical ventilation, like therapy treatment, change for the better the circulation and respiratory system function higher than brain function. "!Ca at the most part at asphyxiated term neonates is a excellent parameters for brain injury. Ultrasound on CNS is separated the asphyxiated group like risk group for periventricular leukomalacio- which in the next period of three to six months development a children with cerebral paralysis. For prediction of neonatal outcome measured as moderate or severe HIE, the sensitivity of S100B > 12 μg/L was 50%, specificity 85%, positive predictive value (PPV) 82% and negative predictive value (NPV) 55%. For prediction of death or development of CP by 3-6 months, the sensitivity of S100 >12 μg/L was 58%, specificity 75%, PPV 50% and NPV 80%. describes the prediction for HIE and outcome of S100B protein> 12 μL/g.

Conclusions:

1. S100B protein is higher at asphyxiated term neonates with high risk for hypoxic ischemic encephalopathy, and S100B protein is a excellent marker for acute brain injury.

2. Mechanical ventilation like therapy treatment change for the better the circulation and respiratory system function higher than brain function.

3. Lower Ca at the most part at asphyxiated term neonates is an excellent parameters for brain injury.

4. Ultrasound on CNS is separated the asphyxiated group like risk group for periventricular leukomalacio- which in the next period of three to six months development a children with cerebral paralysis.

5. For prediction of neonatal outcome measured as moderate or severe HIE, the sensitivity of S100B > 12 μg/L was 50%, specificity 85%, positive predictive value (PPV) 82% and negative predictive value (NPV) 55%.

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