Arq Neuropsiquiatr 2006;64(1):24-29

S100B PROTEIN RELATED NEONATAL HYPOXIA

Régis Osorio Martins[1], Newra Telechea Rotta[2], Luiz V. Portela[3], Diogo O. Souza[4]

ABSTRACT - Biochemical markers have played an increasingly relevant role in the assessment of neonatal asphyxia. The S100B protein is particularly important in research conducted in this field. The purpose of this study was to underline the importance of the S100B protein in the assessment of term newborn infants with hypoxic-ischemic encephalopathy, as well as to relate it to other substances also involved in the ischemic process. An assessment was made from September 2003 to October 2004 of 21 term newborn infants who developed hypoxic-ischemic encephalopathy. Samples were collected on the 1st and 4th day of life and S100B protein and lactate concentrations were calculated using the immune cytochemical method. A positive relationship was found between the two substances. Additionally, a comparison between the two substances showed a statistically significant correlation.

KEY WORDS: S100B, hypoxic-ischemic encephalopathy, newborn, asphyxia.

Relação da proteína S100B com a hipóxia neonatal

RESUMO - A participação de marcadores bioquímicos na avaliação de quadros de asfixia neonatal é cada vez mais relevante. A proteína S100B é de particular importância neste campo. O objetivo deste estudo foi procurar destacar a importância da proteína S100B na avaliação de recém-nascidos a termo com quadro de encefalopatia hipóxico-isquêmica, assim como correlacionar com outras substâncias que também participam do processo isquêmico. Foram analisados 21 casos de recém nascidos a termo que desenvolveram encefalopatia hipóxico-isquêmica no período de setembro de 2003 a outubro de 2004. Realizadas coletas no 1º e 4º dia de vida e dosadas, por método imunocitoquímico, a proteína S100B e o lactato. Observou-se correlação positiva entre as duas substâncias, assim como quando comparadas entre si nas suas respectivas dosagens, obteve-se significância estatística.

PALAVRAS-CHAVE: S100B, encefalopatia hipóxico-isquêmica, recém-nascido, asfixia.

Hypoxic-ischemic encephalopathy (HIE) is a condition with great impact on the body of the newborn 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 areas, 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 prognosis, but not in the first 24 hours, while spectroscopy resonance has cost limitations[1,6-8].

A large number of molecules are asigned 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)[9-12]. Studies that measured the concentration of S100B, the cal-

[1]Master Program Student, Children's Neurologist, Porto Alegre RS, Brazil; [2]Professor of Neurology, Associate Professor Universidade Federal do Rio Grande do Sul (UFRGS) Porto Alegre RS, Brazil; [3]Associate Professor, PhD at the Biochemistry Department, UFRGS; [4]Full Professor, MD, PhD, Biochemistry Department, UFRGS.

Received 6 May 2005, received in final form 24 August 2005. Accepted 18 October 2005.

Dr. Régis Osorio Martins - Rua Alves Nogueira 150 / 403 - 90470-110 Porto Alegre RS - Brasil. E-mail: r.o.martins@terra.com.br

cium-binding protein that prevails in astrocytes, in the CSF and blood, have shown a direct relationship with brain injury[13-15]. 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].

The goal of this study was to check if S100B protein could be used as a brain injury marker in term newborn infants who developed HIE, if its variations show different degrees of involvement and if there is any correlation with other substances, among them, lactate.

METHOD

This study is a longitudinal, prospective analysis of a sample of term newborn infants who had neonatal asphyxia and ensuing HIE born at Hospital Presidente Vargas (HPV) and Hospital de Clínicas de Porto Alegre (HCPA) from September 2003 to October 2004. Premature newborn infants or infants with malformation were excluded from the study.

The studied variables were gender, race, weight, number of gestations, mode of delivery, HIE degree (Sarnat/Sarnat), clinical events and biochemical concentrations of transaminases, CPK, CK-mb and S100B. Results were analyzed using the SPSS 12.0 statistical package.

Peripheral arterial blood was collected on the first and fourth day of life and taken to the UFRGS biochemistry laboratory, centrifuged and stored in a freezer at –70° C until concentration analyses were performed. S100B protein quantification was performed using an immune luminometric assay (BYK - Sangtec, Dietzembach, Germany). This study was approved by the HPV Ethics Committee and by the Research and Graduate Studies Group (GPPG) at HCPA.

Table 1. Variable frequencies.

Variable	n=21	f (%)
Male	12	57.1
Caucasian	15	71.4
Vaginal delivery	13	61.9
Para I	9	42.9
Degree of encephalopathy		
Mild	7	33.3
Moderate	6	28.6
Severe	8	38.1
Death	2	9.5
Seizure	11	52.4

RESULTS

Twenty-one newborn infants with HIE were studied, with peripheral arterial blood collected on the first and fourth day of life. Out of these samples, the second blood collection was not performed in Patient 20, who died on the third day. Seven newborn infants had mild degree HIE, 6 had moderate degree HIE and 8 had severe HIE. Two infants died - 9.5% - both had severe HIE. Caucasian (71.4%) and male (57.1%) subjects prevailed in this sample, both also showing most of the severe HIE cases. Vaginal delivery was the prevailing mode of delivery, with 61.9% (Table 1).

In 8 cases Cesarean section was indicated and severe HIE was found in 50% of them; however, this relationship was not statistically significant (p=0.424). All newborn infants had proper weight for gestational age and mother age varied from 16 to 38 years (average=26±8 years). All mothers reported they performed prenatal care and most of them were para I; ho-

Table 2. Studies variables x degree of encephalopathy.

	Degree of encephalopathy			
Variable frequencies	Mild	Moderate	Severe	P value
Male**	42.9%	66.7%	62.5%	0.742
Caucasian**	57.1%	50%	100%	0.057
Vaginal delivery**	57.1%	83.3%	50%	0.535
Apgar 1'*	3 (2 to 5)	2.5 (0 to 5)	1.5(1 to 3.8)	0.465
Apgar 5'*	6 (6 to 7)	6(4.5 to 6.3)	4.5 (2.3 to 6.8)	0.197
Apgar 10'*	8 (8.0 to 8.8)	7.0 (5.0 to 8.0)	4.5(2.5 to 5.5)	0.015
Seizure**	0	45.5	54.5	0.003

*Asymmetric quantitative variables were compared with K. Wallis, represented by median and interquartile interval (25-75); **Qualitative variables were compared between groups using the Fischer's test and shown in this table as percentages.

Table 3. Analysis of laboratory variables vs degree of encephalopathy.

	Degree of encephalopathy			
Variable	Mild	Moderate	Severe	p value
S1001dv*μg/L	3.7 (1.9–8)	3.7 (2.1–6.2)	4.6 (2.6–7.6)	0.807
S1004dv*μg/L	2.8 (1.5–4.8)	2.0 (1.4–2.4)	1.3 (1.2–2.9)	0.061
CPK*	763.5 (606–1374)	1432.5 (818.5–3150)	839 (243.8–2109)	0.281
CK-mb*	179 (104.6–411)	251 (100.9–615.8)	47 (22.1–597)	0.462
GOT*	71.5 (46.8–140)	107.5 (80.8–415.3)	112 (61.3–715.5)	0.315
GPT*	20 (8–32)	28.5 (26 –194)	24 (19–283)	0.187

*The analysis of assymetric quantitative variables was compared with K. Wallis, represented by median and interquartile interval (25-75).

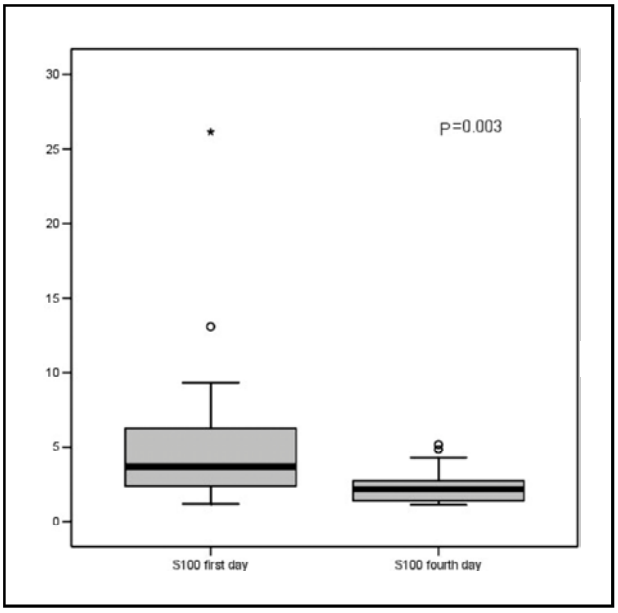

Fig 1. Variation in S100B concentrations (μg/L) in the 1st-4th day.

wever, 30% of the cases did not inform number of visits.

Regarding medical events, seizures should be underlined, since they occurred in 52.4% of the cases. A statistically significant relationship was found between seizures and the severity of HIE (p=0.003). Apgar score at the tenth minute was statistically significant (p=0.015) when compared to the HIE degree (Table 2).

There were no losses in the collections performed in the first day of life for S100B concentrations, while for lactate in 4 samples it could not be performed by hemolysis. In the fourth day, we could not get the concentrations for 2 S100B samples and for 7 lactate samples.

The biochemical analysis made with S100B (1st-4th days of life), CPK, CK-mb, GOT, GPT and lactate (1st-4th days of life), when compared with medical data such as weight, Apgar score, degree of HIE and seizu-

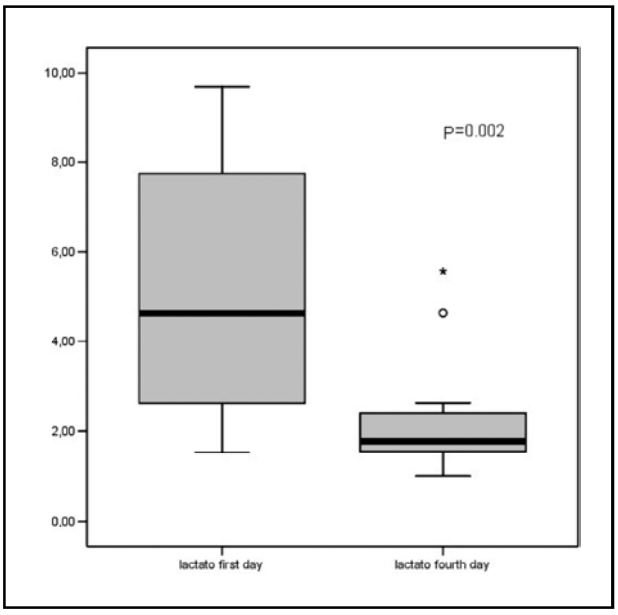

Fig 2. Variation in lactate concentrations (mmol/L) in the 1st-4th day.

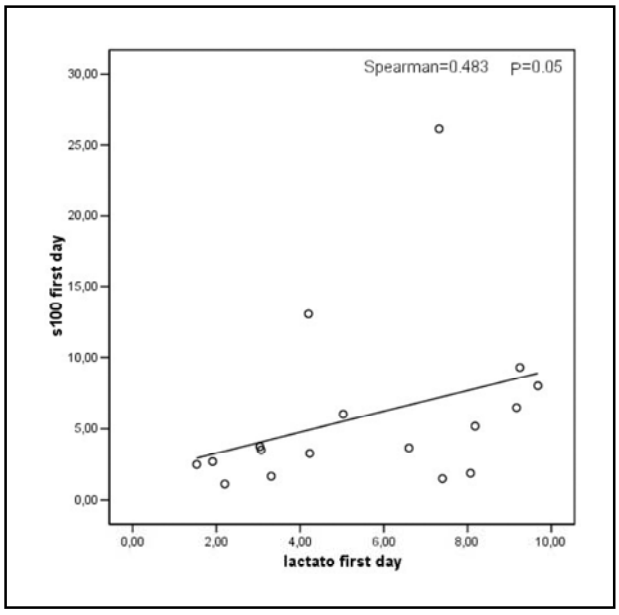

Fig 3. Correlation between S100B concentrations (μg/L) and lactate concentrations (mmol/L) 1st day.

res, was not statistically significant, but were above normal values (Table 3).

Acidosis could be documented in the first 6 hours of life in only 6 newborn infants (28.5%). Nevertheless, a strong association was found between acidosis and S100B and lactate in the first day of life, respectively p=0.029 and p=0.009.

When both substances were compared in their variations, statistical significance was also found in the S100B 1st-4th days group (p=0.003) and in the lactate 1st-4th days group (p=0.002) (Figs 1 and 2). Likewise, a moderate positive correlation was found (rs= 0.483) between S100B and lactate concentrations in the first day (p=0.050) (Fig 3).

No statistically significant relationship was found between S100B and lactate with the other enzymes - GOT, GPT, CPK and CK-mb.

DISCUSSION

Badawi et al. assessed the intrapartum risk factors in newborn infants and their relationship with HIE and found that in relation to vaginal delivery, there was a two-fold increase in risk between Cesarean section and vaginal delivery with the help of instruments and the occurrence of encephalopathy[19]. In a study with 961 low-birth weight newborn infants relating the mode of delivery, cerebral palsy and neonatal death, they found that the only adverse factor related to vaginal delivery were changes in ultrasound findings. No significant association was found concerning the occurrence of cerebral palsy or neonatal death[20].

When assessing the data from this study, we can see that there was a prevalence of vaginal deliveries (61.9%). Out of these vaginal deliveries, only two were performed with the help of instruments and in a third newborn infant a forceps was used, being then subjected to an emergency Cesarean section. These data are similar to those found by Silveira in a study carried out in the same hospital, who found 58% of vaginal deliveries in newborn infants with HIE. On the other hand, the author remarked that mode of delivery did not show any statistically significant relationship with the severity of HIE, which is in agreement with the present research study[21].

Recent studies have shown that the Apgar score, particularly a low one at 5 minutes in term newborn infants, is a specific marker for HIE complications, including seizures[22]. However, seizures in newborn infants with HIE can also be related to metabolic disorders, structural injuries or malformation[1]. In this study, seizures were found in 52.4% of patients and were more frequent in those with Apgar score ≤ 5 at the fifth minute and severe HIE, except for one case with Apgar score 7. The same percentage value was found by Pereira in a study with asphyxiated infants at the same neonatology service[23].

Moster stated in his publication that a low Apgar score at the fifth minute is strongly associated with subsequent death or cerebral palsy[22]. However, the source of many cerebral palsy cases is more related to antenatal events. Australian studies showed that only a small part is actually related to intrapartum hypoxia[7]. Recent research data suggest that, for the most part, neuropathologic findings leading to cerebral palsy occur as a result of multifactorial phenomena that take place both during fetal development and in the neonatal period[2].

Low Apgar scores are not caused by one single adverse condition, because they can be the result of several factors, including intrapartum hypoxia. The persistence of a low Apgar score is most commonly associated with resuscitation effectiveness[3]. The data in this study show that 30% of newborn infants with moderate or severe HIE had an Apgar score of ≥5 at the fifth minute, in agreement with literature data that show Apgar score as a poor predictor of neurological outcome[1].

The literature shows a death rate of 15-20% in neonatal age with the presence of HIE[2]. Newborn infants with an Apgar score of 0-3 at the 5th minute show a relative risk of death = 16 when compared to those with normal Apgar scores[22]. Studies conducted at HCPA by Pereira and Silveira respectively found 35.5% and 15.8% deaths in newborn infants with HIE[21,23], while in this sample the mortality rate was 9.5%. These data show a clear decrease in neonatal mortality in a 4-years period in our setting, reflecting the result of progress in early diagnosis and management of asphyxiated newborn infants.

Thomgren-Jerneck et al. based on clinical and laboratory diagnosis; found 62 term asphyxiated infants in their 4-years study. Their criteria were the following: Apgar <7 at the fifth minute and umbilical artery acidosis (ph <7.10 and/or Base Excess >12)[18]. Although it should be tested in every newborn infant showing signs of asphyxia, metabolic acidemia does not define the time of hypoxia and does not correlate directly with the occurrence of cerebral palsy. When blood gas analysis is not performed in the first hour of life, that hypoxia cannot be considered the causal or contributing factor of the clinical signs that may occur later[1,7,21]. Additionally, many cases of severe neona-

tal encephalopathy are not related to intrapartum hypoxemia[7].

In the analysis of this population-based sample of asphyxiated newborn infants, there were technical difficulties to collect blood at birth to get the umbilical cord pH, which is the most sensitive asphyxia indicator. The fact that the threshold for the blood collection was up to 6 hours after birth led to many of these patients being already under ventilation or drug support, with the consequent change in biochemical results. However, the small number of newborn infants with proven acidosis did not prevent this result from being significant when compared with the biochemical analysis.

One of the main premises in the early assessment of asphyxiated newborn infants is the biochemical analysis of certain molecules. Several substances are considered markers of brain injury[1,10-12]. Animal studies have shown that excitatory amino acids, e.g. glutamate and aspartate, are involved in the pathogenesis of brain injuries and that their agonists are extremely toxic in immature brains[9]. Lactate and lactate/piruvate concentrations can also be used as a parameter of severity in perinatal asphyxia[10]. Other substances are mentioned, e.g. transaminases, NSE, interleukins and ammonia[1,10,12,15].

In the present study a correlation was found with literature data concerning the increase in serum concentrations of the enzymes transaminases, creatinine kinases (CPK) and CK-mb[1], however, this correlation was not statistically significant when compared with the degree of HIE. This is probably due to the small number of cases in this study. However, the study by Nagdyman and coworkers, with 29 cases, showed a significant difference in moderate and severe HIE cases when CK-mb was analyzed in serial collections starting from the 2nd hour of life[12].

The introduction of electrochemical techniques has enabled a fast and accurate determination of the concentration of cellular metabolites important in the biochemical chain of asphyxia, among them piruvate and lactate. Lactate, the end product of anaerobic glycolysis is converted into piruvate when oxygen supply to tissues is restored. Chou et al.[11] showed that determining these concentrations can be an alternative quantitative method to assess hypoxia. When compared with normal reference values of the laboratories of hospitals in which this research was conducted (0.33-1.33 mmol/L), a significant increase in lactate concentrations was found (1.01-9.25 mmol/ L).

The S100B protein is produced and released primarily by astrocytes in the CNS where it has trophic functions on neurons and the glia[24]. Several studies have already shown its role as a peripheral biochemical marker in cases of brain impairment, such as head trauma, stroke, CNS tumors, Alzheimer disease, schizophrenia, systemic lupus erythematosus[13,15,25-27]. However, according to Wijnberger et al. due to the short half-life of S100B (25 min-2 hours) no direct causal relationship can be established with brain injury, except for acute events[17]. Studies with asphyxiated newborn infants or with intraventricular hemorrhage have shown an association with increased S100B concentrations. They have concluded that this protein should be considered as one of the brain impairment markers currently available[12,13,28].

Thomgren-Jerneck et al. found considerably increased S100B concentrations in those term newborn infants who showed moderate and severe HIE, as well as in cases of neonatal death and cerebral palsy up to the 18th month. These authors collected blood samples from the 1st to the 4th day of life and the concentrations in newborn infants with HIE were decreasing, but above average values found for the controls. The authors found a significant correlation between S100B concentrations on the 1st day and acidosis[18].

In the present study, despite the small number of cases, an association was also found between S100B and lactate concentrations on the 1st day with acidosis. This finding enables us to underline the importance of S100B in the anaerobic chain and the resulting cell degeneration, where lactate plays a role. However, no statistically significant correlation was found between S100B concentrations and the degrees of encephalopathy. This finding, which is probably due to the small number of cases, is in disagreement with recent literature findings[12,18]. Additionally, the routine for blood sample collection in this study was not limited to the first 2 hours, which is the time a greater concentration of the S100B protein is found due to its short half-life.

Normal S100B reference values vary with age, being greater at the neonatal period. This fact can be explained by the intense maturation activity of neurons and the glia at this phase of life[14]. Few studies were able to get a S100B concentration curve related to time of collection that would allow us to find the right time for its detection and, as a consequence, greater reliability as a biochemical marker. The authors stress that repeated collections for concentration determination should be useful to predict brain damage that might occur[12,14,18].

When comparing the values found in the S100B samples in this study, we can see a significant difference between samples collected on the 1st and 4th day of life. This situation is similar to those reported in the literature[18]. It is clear that the possibilities of neuroprotective interventions are progressively increasing for all ages. It is necessary to recognize those patients who will benefit from such interventions. Probably the study of several substances, which together could lead to an early indication of brain injury signs, would be the starting point to understand neuroprotection. For this purpose, S100B protein has a promising future, provided it is associated with other biochemical markers and clinical data.

REFERENCES

1. Rufo-Campos M, Palencia-Luaces R. Encefalopatia hipóxico-isquémica del recien nacido a término: recientes avances, marcadores de hipoxia y opciones terapêuticas. Rev Neurol 2000;31:617-623.
2. Volpe J. Neurology of the newborn, unit III, 4th edition, chapter 6-9, 2001.
3. Ferriero DM. Neonatal brain injury. N Eng J Med 2004;351:1985-1995.
4. Macaia A. Muerte celular en la hipoxia-isquemia neonatal. Rev Neurol 2000;31:784-789
5. Grow J, Barks JDE. Pathogenesis of hypoxic-isquemic cerebral injury in the term infant: current concepts. Clin Perinatol 2002;29:585-602.
6. Sarnat HB, Sarnat HS. Neonatal encephalopathy fetal distress: a clinical and eletroencephalographic study. Arch Neurol 1976;33:696-705.
7. MacLennam A. A template for defining a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. BMJ 1999;319:1054-1059.
8. Thorngren-Jerneck K, Ohlsson T, Sandell A, et al. Cerebral glucose metabolism measured by positron emission tomography in term newborn infants with hypoxic isquemic encephalopathy. Pediatr Res 2001; 49:495-501.
9. Hagberg H, Thornberg E, Blennow M, et al. Excitatory amino acids in the cerebrospinal fluid of asphyxiated infants: relationship to hypoxic-ischemic encephalopathy. Acta Pediatr 1993;82:925-929.
10. Esqué-Ruiz MT, Figueras-Aloy J, Salvia-Roigés MD, Carbonell-Estrany X. Amoníaco y transaminasas en sangre en el recién nacido a término afectado de asfixia perinatal. Rev Neurol 2003;36:801-805.
11. Chou YH, Yau TI, Wang PJ. Clinical application of the measurement of cord plasma lactate and pyruvate in the assessment of high risk neonates. Acta Paediatr 1998;87:764-768.
12. Nagdyman N, Wolfgang K, Ko HK, Muller C, Obladen M. Early biochemical indicators of hypoxic-ischemic encephalopathy after birth asphyxia. Pediatr Res 2001;49:502-506.
13. Whitelaw A, Rosengren L, Blennow M. Brain specific proteins in posthaemorrhagic ventricular dilatation. Arch Dis Child Fetal Neonatal 2001;84:F90-F91.
14. Portela LV, Tort ABL, Schaf DV, et al. The serum S100B concentration is age dependent. Clin Chem 2002;48.
15. Berger RP, Pierce MC, Wisniewski SR, et al. Neuron specific enolase and S100B in cerebrospinal fluid after severe traumatic brain injury in infants and children. Pediatrics 2002;109:1-6.
16. Gazzolo D, Bruschettini M, Di Iorio R, et al. Maternal nitric oxide supplementation decreases cord blood S100B in intrauterine growth-retarded fetuses. Clin Chem 2002;48:647-650.
17. Wijnberger L, Nikkels PGJ, Angelique JCM, et al. Expression in the placenta of neuronal markers for perinatal brain damage. Pediatr Res 2002; 51:492-496.
18. Thorngren-Jerneck K, Alling C, Herbst A, Amer-Wahlin I, Marsal K. S100 protein in serum as a prognostic marker for cerebral injury in term newborn infants with hypoxic ischemic encephalopathy. Pediatr Res 2004;55:406-412.
19. Badawi N, Kurinczuk JJ, Keogh JM, et al. Intrapartum risk factors for newborn encephalophaty: the Western Australian case control-study. BMJ 1998;317:1554-1558.
20. Qiu H, Paneth N, Lorenz JM, Collins M. Labor and delivery factors in brain damage, disabling cerebral palsy and neonatal death in low- birth weight infants. Am J Obstet Gynecol 2003;189:1143-1149.
21. Silveira RC. Níveis plasmáticos e liquóricos de interleucina-6 e fator de necrose tumoral alfa em recém-nascido a termo com encefalopatia hipóxico-isquêmica. Tese de Doutorado. Porto Alegre, 2003.
22. Moster D, Lie RT, Irgens LM, Bjerkedal T, Markestad T. The association of Apgar score with subsequent death and cerebral palsy: a population-based study in term infants. J Pediatr 2001;138:798-803.
23. Pereira DN. Efeitos da asfixia perinatal sobre a tireotrofina (TSH) e os hormônios da tireóide. Tese de Doutorado. Porto Alegre, 2000.
24. Donato R. Intracellular and extra cellular roles of S100 proteins. Micr Res Tech 2003;60:540-551.
25. Ortiz-Muñoz B, Menéndez-López A, Yayá-Tur R, Arribas-Alpuente L, Maiquez-Richart J, Bordes-Monmeneu M. Proteina S100 en tumores del sistema nervioso central. Rev Neurol 2003;36:1011-1115.
26. Manev H, Manev R. S100B: an old neurotrophic factor with putative new roles in psychiatric illnesses. J Psychiat Res 2001;35:347-348.
27. Lara DR, Gama CS, Belmonte de Abreu P, et al. Increased serum S100b protein in schizofrenia: a study in medication-free patients. J Psychiat Res 2001;35:11-14.
28. Blennow M, Savman K, Ilves P, Thoresen M, Rosengren L. Brain-specific proteins in the cerebrospinal fluid of severely asphyxiated newborn infants. Acta Pediatr 2001;90:1171-1175.