Study of Serum Markers of Brain Injury as Early Predictors of Neonatal Hypoxia/Ischemia

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Abstract:

Neonatal hypoxia-ischemia remains a frequent cause of cerebral palsy, mental retardation, learning disability, and epilepsy. HIE must be identified as soon after birth as possible so that appropriate measures could be taken on arrival in the neonatal intensive care unit. The aim of this study was to investigate the postnatal levels of markers of brain injury, which are CK BB and Protein S-100B in serum and to determine whether hypoxic-ischemic brain damage alters these markers and whether HIE can be predicted by elevated serum concentrations soon after birth. We have included 20 neonates with HIE together with 15 control neonates in our study. Serum concentrations of CK BB and protein S-100B were determined after birth and 24 hours of age. Our results demonstrated that cases with HIE had higher values of cord and 24 hours blood levels of CK BB, and higher values of cord and 24 hours levels of protein S-100B, and when doing statistical analysis to compare these results with those of control group, this difference was significant in all except cord level of protein S-100B. we conclude from our results that CK BB and protein S-100B are predictive of HIE in full term neonates when measured soon after birth, yet the decision as to which infants could be candidates for postasphyxial measures should probably be based on several findings, which include cord blood pH, Apgar score, and serum protein S-100 and CK-BB. Future work to establish the predictive value of these markers in long-term brain injury in neonates is recommended.

Abbreviations:

CK BB = creatine phosphokinase brain specific, PS-100 B = protein S-100B, HIE = hypoxic ischemic encephalopathy.

Introduction:

Perinatal cerebral hypoxia-ischemia remains a frequent cause of the chronic handicapping conditions of cerebral palsy, mental retardation, learning disability, and epilepsy. Fifteen to 20% of such asphyxiated infants who exhibit hypoxic-ischemic encephalopathy actually die during the newborn period, and of the survivors, 25% will exhibit permanent neuropsychological deficits.[1]

Neonates subjected to transient hypoxia-ischemia during an episode of birth asphyxia seem relatively normal soon after resuscitation, but show evidence of delayed cerebral injury some hours later. The mechanism of delayed injury is still unclear, but in the brains of infants dying after birth asphyxia, cells can be detected which show the hallmarks of apoptotic death.[2]

Energy substrates in the neonatal brain continue to run down for 12 to 48 hours after hypoxia, therefore an intervention might be effective 2 to 6 hours after birth asphyxia.[3] The therapeutic window is that interval after resuscitation from hypoxia-ischemia, during which an intervention might be effective in reducing the severity of the ultimate brain damage. The therapeutic window in adults can extend for several hours to a day or more.[4,5] Such is not the case in perinatal animals and presumably in human infants, in whom the process of cellular destruction is much more rapid than in adults. Accordingly, in the full-term infant, the therapeutic window would be short and possibly no longer than 1 to 2 hours.[1]

Given the presumed short therapeutic window, such infants must be identified as soon after birth as possible so that appropriate measures are taken on arrival in the neonatal intensive care unit. Clinical investigations suggest that infants at highest risk for hypoxic-ischemic brain damage include those who have exhibited progressive fetal heart rate abnormalities shortly before birth, are severely depressed at birth (very low Apgar scores), exhibit an acidosis with a pH < 7.0 on umbilical cord blood oxygen and acid-base analysis, and require major resuscitation in the delivery room, including cardiac massage and intubation.[6,7]

Cranial tomography, somatosensory evoked potentials, and magnetic resonance tomography are useful for prognosis, but not in the first 24 hours after birth.[8,9] Several studies measured biochemical factors in serum and cerebrospinal fluid: glial fibrillary acidic

protein after 12–48 hours;[10] excitatory amino acids after 18–66 hours;[11] and IL-6 12 hours after the hypoxic-ischemic event.[12] Urinary lactate:creatine ratio predicts HIE within 6 hours with ^{1}H nuclear magnetic resonance spectroscopy,[13] but a useful indicator for HIE should be specific even earlier, and requires a rapid and readily available laboratory technique. Besides this, infants with asphyxia often have oliguria, and urine sampling may not be possible.[14]

CK-BB is found in both neurons and astrocytes. CK-BB levels were found to be elevated after birth asphyxia, but few data exist concerning the early time course of these factors after the hypoxic-ischemic event.[15-17] In addition, little information concerning this marker, CK BB, in neonatal HIE are available in our area.

Recently, protein S-100B has been found to be a promising marker for central nervous system injury.[18-25] Protein S-100B is part of a large family of calcium-binding proteins, and evidence exists that S-100 regulates calcium-dependent cellular signaling in neuronal differentiation, outgrowth, and apoptosis.[26-28] Elevated levels of S-100B have been observed after focal ischemia in cerebrospinal fluid,[29] and also in serum.[20-24]

Protein S-100B is highly specific for the assessment of astroglial injury. Astroglial cells are the most common cells in the brain. They constitute a supporting framework for neurons. Astroglial cells are known to be as sensitive as neurons to hypoxic stress. Therefore, a marker for astroglial cell damage may indirectly reflect neuronal damage.[18-20,30,31]

Our objectives were to investigate the postnatal levels of these markers, CK BB and Protein S-100B in serum and to determine whether hypoxic-ischemic brain damage alters these markers and whether moderate or severe HIE can be predicted by elevated serum concentrations soon after birth.

Subjects and Methods:

Twenty full-term newborn infants (gestational age 37–42 week) who fulfilled the following criteria were included in the study: arterial blood cord pH value <7.0 or arterial cord blood pH value between 7.01 and 7.1 and also an Apgar score after 5 min of <7. Fifteen full-term infants who fulfilled all the following criteria were enrolled in the study as control group: no maternal illness, arterial blood cord pH ≥7.2, after 5 minutes an Apgar score of >7, and an uneventful course during postnatal life. Predefined exclusion criteria for both groups were congenital anomalies, tumors, maternal drug addiction, severe infections, and congenital metabolic disorders.

Neurological examination was performed. Mild HIE was assumed according to Sarnat and Sarnat if hyperexcitability or hypotonia persisted without seizures for ≥72 h after birth; moderate if the newborn was lethargic, had hypotonia, weak primitive reflexes, and seizures; and severe if the infant had apnea, flaccid weakness, frequent seizures, or coma.[32]

After admission to the neonatal intensive care unit, blood samples were collected from cord blood and 24 hours after birth. Immediately after sampling, the blood in the tubes was placed on a mixture of water and ice to ensure a constant temperature of ≤4°C. The tubes were centrifuged within 1 hour of collection. The plasma was then separated and stored in plastic tubes at -60°C until it was assayed.

Serum S-100 protein was measured with an immunoradiometric assay kit (Sangtec S-100, IRMA, Sangtec Medical). Each patient's sample (100 µL) and diluent (100 µL; phosphate buffer with bovine serum albumin) were incubated with a plastic bead coated with monoclonal antibody to S-100 for 1 hour at room temperature on a shaker. The bead was then washed to remove unbound material and incubated with 200 µL of tracer (^{125}I-labeled monoclonal antibody to S-100) for 2 hours. After unreacted radioactive antibody was washed off the bead, the radioactivity bound to the bead was measured with a gamma counter. The minimum measurable S-100 value was 0.3 µg/L. Creatine kinase was determined at 25°C according to the optimized German standard method on Dax 72 (Bayer, Munich, Germany) analyzer. To quantify CK-BB, creatine kinase isoenzymes were fractionated electrophoretically on agarose gels, visualized by in-gel substrate reaction for fluorometric scanning using Helena (Greiner, Flacht, Germany) gel kits and rapid electrophoresis system.

For statistical analysis, the SPSS 7.5 for Windows (SPSS, Chicago, IL, U.S.A.) was used. Probability values <0.05 were considered to be significant.

Results:

Twenty full term neonates were enrolled, no one was excluded from the study, 15 control neonates were selected and sampled in the course of routine neonatal screening. This work was done in a tertiary care private hospital with a neonatal intensive care facility.

Table I demonstrates some characteristics of study cases: both groups were comparable as regards birth weight and gestational age. There was difference between the two groups as regards Apgar score at 1 and 5 minutes and cord pH, and this difference was of high statistical significance.

Table II compares both study groups as regards cord and 24 hours levels of CK BB, and cord and 24 hours levels of protein S-100B, it was found that there was difference between both groups as regards cord and

Table I: Characteristics of study group and control group: birth weight, gestational age, Apgar score, and cord blood pH, together with comparison between means of these characteristics in both groups

	Study groups	Number	Mean	Std. Deviation	t value	Significance
Birth weight in gms	Hypoxia ischemia grp	20	3170.00	465.21	1.62	.16 #
	Control grp	15	3406.67	372.67		
Gestational age in wks	Hypoxia ischemia grp	20	38.70	1.59	.38	.71 #
	Control grp	15	38.93	2.05		
Apgar score at 1 min	Hypoxia ischemia grp	20	4.20	1.44	9.00	.000 **
	Control grp	15	7.87	.74		
Apgar score at 5 min	Hypoxia ischemia grp	20	6.00	.97	10.90	.000 **
	Control grp	15	9.20	.68		
Cord blood pH	Hypoxia ischemia grp	20	6.98	1.79	10.93	.000 **
	Control grp	15	7.24	1.41		

** significant at p < .01, ** highly significant (p < .001), # non-significant , St dev = standard deviation*

Table II: comparison of both study groups as regards cord and 24 hours level of CK BB, and cord and 24 hours level of protein S-100B

Serum marker	Hypoxia/Ischemia grp (n=20)	Control grp (n=15)	t value	Signif.	95 % C.I. of the mean
Cord blood CK BB (in U/L)	Mean ±SD = 24.30 ± 12.88	Mean ±SD = 13.27 ± 9.92	2.76	.009*	(2.89–19.17)
24 hours blood CK BB (in U/L)	Mean ±SD = 19.20 ±11.06	Mean ±SD = 9.13 ± 6.75	3.11	.004*	(3.48-16.65)
Cord level of protein S (in µg/L)	Mean ±SD = 1.29±.75	Mean ±SD = 1.18 ±.49	.47	.64 #	(.35 - .56)
24 hours protein S (in µg/L)	Mean ±SD = 2.99 ±1.84	Mean ±SD = 1.25 ±.53	3.54	.001*	(.74 – 2.74)

** significant at p < .01, N = number of cases, SD = standard deviation, C.I confidence interval*

Table III: Regression model using cord pH as dependent variable, and using serum markers as independent variables

Serum markers	Regression coefficient	t value	Significance
Cord blood CK BB (in U/L)	-3.99	-1.377	.179 #
24 hours blood CK BB (in U/L)	3.45	.084	.933 #
Cord level of protein S (in µg/L)	-7.42	-.021	.983 #
24 hours protein S (in µg/L)	-5.21	-4.282	.000 **

*** highly significant (p < .001), # non-significant*

24 hours CK BB, and this difference was statistically significant. In addition, there was statistically significant difference between both groups as regards 24 hours level of protein S-100B. Difference between both groups as regards cord level of protein S-100B was not significant statistically.

Table III demonstrates regression model using cord pH as a parameter of hypoxia/ischemia in our study cases as dependent variable. Serum markers: cord and 24 hours CK BB levels and cord and 24 hours protein S-100B levels were used as independent variables. It was found that only 24 hours level of protein S-100B is significantly predictive of lower pH in study cases, whereas the other variable were not significantly predictive of lower cord pH in neonates.

Discussion:

Perinatal asphyxia is a common cause of neonatal morbidity and mortality and neurologic disabilities among survivors. In addition to pulmonary, renal, and cardiac dysfunction, HIE develops in one third of asphyxiated newborns.[33] Mild encephalopathy carries a good prognosis, although in moderate and severe encephalopathy the risk of death or neurologic sequelae increases greatly.[34]

Global interruption of the cerebral circulation causes general brain edema and selective neuronal death in vulnerable areas of the brain, and extended anoxia time leads to infarctions in cortical and subcortical regions.[35,36]

In the last years, new diagnostic technologies were developed to assess brain development and to identify early brain injury. Some of them are very attractive methods but invasive, expensive, and time-consuming. The availability of clinically useful serum markers of risk for perinatal brain damage will easily permit the development of rational strategies for prevention of cerebral insults in neonates and more accurate prognostic counseling.

This study was carried out in the purpose of detecting levels of 2 markers in serum : one relatively known before which is CK BB, and the other is a recent marker which is protein S-100B, these markers were studied in neonates with hypoxia/ischemia, together with control neonates. Our results showed that both study groups are matched as regards birth weight and gestational age as shown in table I. When comparing both groups as regards Apgar score and pH there was highly statistically significant difference, which is expected in view of our selection of cases as hypoxic/ischemic group with lower pH values due to concurrent acidosis, together with lower Apgar scores than neonates of the control group.

Table II demonstrates that our cases had higher values of cord and 24 hours blood levels of CK BB, and higher values of cord and 24 hours levels of protein S-100B, and when doing statistical analysis this difference was significant in all but cord level of protein S-100B. CK-BB release comes only from the brain. About this, it might be a simple phenomenon of general ischemia related to asphyxia. Asphyxia may involve the whole body, and the release of proteins into the blood might be a general sign of change in cell membrane integrity and vascular permeability caused by the whole body ischemic-reperfusion injury. It was found before that elevated serum levels of S-100 have been documented in patients suffering from different types of brain damage, including stroke, minor and severe head injury, and brain damage associated with extracorporeal circulation and later stages of global cerebral ischemia.[18-25] The presence of S-100 in serum indicates cellular brain injury and damage to the blood-brain barrier.[26-31]

Table III demonstrates regression analysis with cord pH as dependent variable and our studied markers as independent variables. In this model, there was predictive ability of 24 hours level of protein S100B to detect hypoxia, but not the other levels. This could be explained in part that other clinical and laboratory parameters are taken into consideration in table II so there is no discrepancy between results of table II with those in table III.

Previous studies are in accordance with our results. In the work of Nagdamyan et al.,[37] it was found that the combination of CK BB and protein S-100B detection after 2 hours of birth in neonates had the highest predictive value of predicting moderate or severe HIE as described by the authors. Their work differs from ours in sampling time and statistical analysis but results are matched except cord level of protein S-100B which was higher in HIE group but not to the level of statistical significance when compared with control group in our study.

In one study, Serum CK-BB activity was studied on the first day of life in asphyxiated infants and infants born after high-risk pregnancies (pre-eclampsia or intrauterine growth retardation, or both). CK-BB activity after birth was found to be predictive of neonatal death but not of neurological damage in survivors.[38] Another study to assess whether plasma CKBB levels or Sarnat scores are more accurate for prediction of poor neurological outcome in babies with suspected birth asphyxia concluded that CKBB was elevated following birth asphyxia but was a poor predictor of adverse neurological outcome.[39]

In another work, the concentration of CK-BB was measured in blood in children (less than 1 year old) with congenital heart disease. CK-BB levels were significantly higher than in children without cyanosis, and negative correlation was found for CK-BB concentration and arterial oxygen saturation. It was suggested that the increased CK-BB levels in the blood of cyanotic children reflect chronic cerebral hypoxia.[40] Study population is different from ours yet results are in accordance with ours.

Another study was aiming at evaluating CK isoenzymes (CK-MM, CK-MB, and CK-BB) in umbilical cord blood sera of newborns in relation to their acid-base status. There were no significant differences in total CK, CK-MM and CK-MB activities in examined groups of newborns. They found a significant rise of CK-BB activity in cord sera of hypoxic infants.[41]

In another work, serum CK BB activities were measured in asphyxic full-term infants. The infants were followed for a mean period of 16 months. Enzyme activities at 4 hours of life were significantly higher in those neonates who died of severe hypoxic-ischemic encephalopathy or developed neurological sequelae than in those who did not present neurological abnormalities during the follow-up time. CK BB was described by the author as of limited value to predict the neurological outcome after neonatal asphyxia.[15]

Another study measuring CK-BB activity showed increased levels after hypoxic conditions associated with central nervous system disease. Newborn babies were studied with serial measurements of CK-BB activities during the first three postnatal days. These values were correlated with their early neurologic outcome at 5 months of age. The author concluded from his work that early CK-BB determinations could be used as indicator of neonatal brain damage.[42]

Another study concluded that a high level CK BB in blood of newborn infants with perinatal brain damage had an accurately diagnostic value, if the blood puncture is done immediately during the severe CNS damage as described by the authors.[43]

Another work found that infants with severe asphyxia and neurologic damage had a significant rise in serum CK-BB. When the peak CK-BB level exceeded 35 IU/liter, the mortality was high (83%).[44]

In another work, CK BB was determined in cerebrospinal fluid in preterm neonates. Neonates with HIE stages II and III showed markedly higher CK-BB values than those with HIE I on day 2 and day 5 of life. CK-BB values were markedly higher in preterm babies with none of some primitive responses as described by the investigator.[45] We did not include preterms in our study yet results are in accordance with ours.

Another study was carried out in newborns to evaluate the accuracy of 1-minute Apgar score and

umbilical arterial pH for prediction of the risk of perinatal brain damage, using CK-BB determination. Patients with low Apgar score at one minute of life had significantly higher cord blood CK-BB values than the control group.[46]

In another study, serum protein S-100B and CK-BB sampled on the first day of life were found to be of limited value in predicting severe brain damage after birth asphyxia.[47] This was not the aim of our study to examine long-term effects of HIE, so this can be a future extension of our work.

In one study, protein S-100 serum levels in healthy newborns during the first week of life and newborns with perinatal acidosis were measured. Newborns with signs of hypoxic-ischemic encephalopathy (HIE) after perinatal acidosis showed elevated Protein S-100 serum levels, whereas newborns without these signs had normal concentrations.[48]

In one work, protein S-100 was measured in a group of preterm infants suffering perinatal asphyxia. The results of this study showed significantly higher protein S-100 serum levels in asphyxiated preterm babies with a peak at 24 hours of life compared with healthy preterm babies. These data suggest that elevated protein S-100 serum levels can be considered as an indicator of regional brain damage in preterm infants as concluded by the authors.[49] Our results are in accordance with this work but sampling time and study population (preterm versus full term) was different from ours.

In another work, significantly elevated S-100B serum levels 12 hours after cardiac arrest correlated well with an unfavorable neurologic outcome after 12 months.[50] Their population of study was different from our target population, but still results are in accordance with ours. Also in another study in adults protein S-100B was found to be able to assess the extent of primary brain damage after trauma.[51]

Conclusion:

Serum markers of brain injury as CK BB and protein S-100B are predictive of HIE in full term neonates when measured soon after birth. Yet the decision as to which infants could be candidates for postasphyxial treatment should probably be based on several findings, which include cord blood pH, cord blood base deficit, Apgar score, as well as serum protein S-100B and CK-BB. These biochemical markers may be helpful in deciding whether an early initiated neuroprotective therapy should be continued or stopped. Future work to establish the predictive value of these markers in long-term brain injury in neonates is recommended.

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