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Hypoxic-Ischemig Encephalopathy in Term Neonates: Early Biochemical Indicators

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Abstract: Hypoxic-ischemic encephalopathy (HIE) after perinatal asphyxia is a condition in which serum concentrations of brain-specific biochemical markers may be elevated. Neuro-protective interventions in asphyxiated newborns require early indicators of brain damage to initiate therapy. Our aim is to investigate serum concentration of brain-specific biochemical markers, as early biochemical indicators of neonatal asphyxia. The study was carried out at the Neurology, Pediatric and Clinical Pathology Department, Zagazig and Al-Azhar Universities Hospitals. It was conducted on 30 infants with perinatal asphyxia. We examined brain-specific creatinekinase (CK-BB), protein S-100 and neuro-specific enolase (NSE) in cord blood and at 2,6,12 and 24 h afterbirth. At 2 h afterbirth, median (quartiles) serum CK-BB concentration was 16.0 U/L in infants with mild HIE and 36 U/L in infants with moderate HIE and 46.5 U/L in infants with "severe HIE. Serum protein S-100 2 h afterbirth was 2.9 ug/L in asphyxiated infants with mild HIE, 3.9 ug/L in infants with moderate HIE and 17.9 ug/L in infants with severe HIE while no significant difference was detectable in serum neuro-specific enolase between infants with mild, moderate and severe HIE 2 h and 6 h afterbirth. A combination of serum protein S-100 (cutoff value, 8.5 ug/L) and CK-BB (cutoff value, 18.8 U/L) 2 hr after birth had the highest predictive value (83%) and specificity (95%) of predicting moderate and severe HIE. Cord blood pH (cutoff value, < 6.9) and cord blood base deficit (cutoff value, > 17mM/L) increase the predictive values of protein S-100 and CK-BB. We conclude that elevated serum concentrations of CK-BB and protein S-100 reliably indicate moderate and severe HIE as early as 2 h afterbirth.

Key words: Neonatal-Hypoxic-ischemic encephalopathy-CK-BB-Protein S-100-NSE Creatinekinase.

INTRODUCTION

Perinatal asphyxia is a common cause of neonatal morbidity and mortality and neurologic disabilities among survivors. HIE develops in one third of asphyxiated newborns (Good- win *et al*., 1992). Neuro-protective interventions are increasingly in the forefront of interest and have been shown to be effective. For clinical intervention, it is important to identify infants at a high risk for brain damage soon after birth and within the therapeutic windows (Vannucci and Perlman, 1997). Several indicators of brain damage have been investigated in the last decade (4-10). Early recognition of HIE is important in guiding the management of these neonates and justifying administration of certain drugs, proposed as Neuro-protectors.

The objective of this work is to investigate the postnatal time course of these markers (brain-specific creatinekinase (CK-BB), protein S-100, and neuron-specific enolase) 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.

Patients and Methods:

A written informed consent was given by the parents (Thirty infants with perinatal asphyxia). The protocol included 30 full-term newborn infants (gestational age, 39-42 wks) who fulfilled the following criteria were included in the study: arterial blood cord pH < 7.0, or arterial blood cord pH value between 7.01 and 7.1 and also an Apgar score after 5 min of < 7. The asphyxia group was subdivided according to the clinical examination: mild HIE with a good prognosis, moderate and severe HIE with a greater risk of neural handicap (18).

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Study Design:

Blood samples were collected from cord blood and 2, 6, 12 and 24 h afterbirth. Standardized neurologic examinations were performed at 6, 12, 24, 48, and 72 h of age. Mild HIE was assumed according to Sarnat (19) mild 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.

Analysis of CK-BB, protein S-100 and NSE Creatinekinase was determined at 25 Co according to the optimized German standard memo! on Dax 72 (Bayer, Munich, Germany) or Modular PP (Roche, Mannheim, Germany) random assessment clinical analyzers. To quantify ' CK-BB, creatinekinase isoenzymes (Delivoria *et al*., 1998) 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. The area under the CK-BB curve was used to calculate its concentration.

Protein S-100 was measured manually with a sandwich type immuno-Iuminometric assay kit (Byk Sangtec, Dietzenbach, Germany) that used MAb and an LB952 luminometer (Berthold, Wildbad, Germany). The assay uses three MAbs to detect the B chains in the BB(S-I00B) and B(S-l00AL) dimers. We have used the term protein S-100 for simplicity, which refers to both of these dimers.

NSE was measured on a Cobas Core II immunoanalyzer with the NSE ELISA II kit (Roche), a one-step sandwich type enzyme immunoassay that used two specific mouse MAbs. Free Hb, as an indicator of hemolysis, was quantified by bichromatic photometric measurement on Hitachi 911 or Modular PP analyzers (Roche). The manufacturer claims low detection limits of 0.I ug/L for MSB and 0.02 ug/L for protein S-100. In 30 cases of low sample volumes, we reduced the amount of serum for protein S-100 assay from 100 uL to 25 uL. and 0.08 ug/L as the lower detection limit. Least square regression analysis for 18 samples were performed with both the original and the diluted protocol, and gave y (25 uL) - 1.019 X (100 uL) - 0.04 with a regression coefficient of 0.98. Time required for the measurement of CK-BB, protein S-100, and NSE was 0.5, 3.5 and 0.75 h, respectively.

Data Analysis:

All values were presented as medians and interquartile ranges. Group comparisons were performed with the Mann-Whitney U test. Positive predictive value, negative predictive value, sensitivity, and specificity I; for development of moderate or severe asphyxia were obtained using optimal cutoff levels and were calculated on the material used in our study. Receiver operating characteristic curves were assessed using the areas under the curves. Correlations were calculated by the Spearman rank method. Probability values <0.05 were considered to be significant.

Results:

Table 1: Clinical characteristics of the studied asphyxiated neonates with different stages of HIE and control group.

Clinical characteristics	Stage I HIE (No. 12)	Stage II HIE (No. 9)	Stage III HIE (No. 11)
Sex (M/F)	9/3	7/2	8/3
Gestational age (wks)*	41.7 ± 1.6	40.9 ± 0.9	39.6 ± 1.7
Birth weight (gms)*	3350 ± 516	3409 ± 707	3695 ± 406
Apgar score**			
-Ist min.	2 (1-3)	5 (4-6)	1 (1-2)
-5th min.	5 (l-6)	1 (0-3)	2 (0-5)
No. of infants needing Ambu bagging ≥ 3 min.	10	8	8
No. of infants needing external cardiac compression and medications	0	2	6
No. of infants needing long-term mechanical ventilation ≥ 24h.	0	2	6

*Values are given as mean ± SD; **Apgar scores are recorded as median (minimum-maximum).

Table 2: Serum concentrations of CK-BB, protein S-100 and NSE at different times.

Biochemical markers (median/interquartiles)	Stage 1 HIE	Stage 11 HIE	Stage III HIE	P value*	P value
		CK-BB (U/L) :			
Cord blood	24.5 (7.5-48.5)	26.5 (17.5-58.5)	15.0 (14.0-40.0)	0.005	NS
2h	16.0 (13.0-23.5)	36.0 (19.5-26.7)	46.5 (21.4-83.5)	O.OOO	0.003
6h	10.4 (6.0-16.0)	20.8 (8.0-26.0)	27.4 (18.0-56.0)	I 0.002	0.002
12 h	6.5 (4.0-14.0)	21.5 (7.0-19.6)	33.5 (17.1-52.6)	<0.0001	0.012
24 h	12.5 (6.0-15.6)	9.6 (6.0-17.0)	9.6 (7.0-17.5)	0:028	NS

Table 2: Continue.

		Protein S-l 00 (ug/L):			
Cord blood	1.5 (1.1-1.9)	2.6 (2.1-6.9)	2.6 (2.1-6.9)	0.000l	NS
2h	2.9 (1*8-4.7)	3.9 (3.5-5.4)	17.9 (3.2-35.1)	<0.0001	0.008
6h	2.5 (1.6-3.8)	4. 5 (2.4-5.9)	27.6 (2.6-52.3)	0.001	0.015
12 h	1.8 (1.5-2.3)	2.3 (2.5-5.9)	3.1 (1.5-23.1)	0.00	NS
24 h	1.6 (1.0-2.6)	1.9 (1.8-3.7)	3.9 (1.2-9.7)	1 0.04	NS
		NSE (ug/L):			
Cord blood	48.9 (20.1-74.7)	51.8 (29.3-82.6)	106.8 (60.5-108.1)	NS	NS
2h	32.5 (20.8-60.7)	42.5 (28.8-65.1)	60.8 (49.3-89.1)	NS	NS
6h	35.6 (30.1-70.3)	39.5 (35.2-72.4)	52.7 (48.7-79.3)	NS	NS
12h	34.0 (24.6-48.7)	36.0 (25.3-50.9)	54.3 (46.8-78.8)	NS	0.028
24 h	33.1 (23.5-52.1)	31.2 (26.5-57.4)	51.9 (24.5-67.9)	NS	NS

P* values are for comparison between control infants (No=20) and infants with asphyxia (No=30) Pf values are for comparison between infants with mild HIE , moderate and severe HFE.

Table 3: Values for predicting moderate or severe HIE.

Variable	Cut off value	PPV (%)	NPV (%)	Sens (%)	Spec (%)	AUC
			CK-BB:			
2h	18.8 U/L	46	100	100	65	0.879
6h	17.0U/L	55	94	86	77	0.877
			Protein s-100:			
2h	8.5 ug/L	71	90	71	90	0.832
6h	4.6 ug/L	63	90	71	86	0.805
			NSE:			
2h	44ug/L	46	93	83	68	0.768
6h	46ug/L	42	93	83	65	0.763
Arterial cord blood $_{p}$H	<6.9	46	89	71	73	0.838
Cord blood base deficit	>17mM/L	50	94	83	77	0.905
Apgar score (Imin)	<3	50	94	73	86	0.825

Abbreviation: PPV, positive predictive value; NPV, negative predictive value; Sens., sensitivity, Spec, specificity; AUC, area under the curve.

Table 4: Combination of factors for predicting moderate or severe HIE.

Variable	PPV (%)	NPV (%)	Sens (%)	Spec (%)
CK.-BB and Protein S-100	83	91	71	95
CK-BB and cord blood pH	71	91	71	91
CK-BB and cord blood base deficit	67	91	67	91
CK-BB and Apgar score- (Imin)	83	96	83	95
Protein S-100 and cord blood pH	100	88	57	100
Protein S-100 and cord blood base deficit	100	92	67	100
Protein S-100 and Apgar score (1 min)	80	88	57	96

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; Sens.; sensitivity; spec and specificity. Values of serum factors are at 2 h after birth.

Discussion:

Neonatal asphyxia and hypoxic-ischemic encephalopathy frequently result in neurologic injury and Neuro-developmental delay e.g., cerebral palsy, learning disabilities, epilepsy or even mental retardation (Volpe, 1995). A complex and interrelated alterations are postulated for understanding the pathogenesis of hypoxic-ischeinic encephalopathy (HIE), although the disturbed metabolism seems to have a key role in neuronal damage (Distefano and Pratic, 2010). Early recognition of the hypoxic- ischemic injury is important in guiding the management of these neonates and justifying administration of certain drugs, proposed as "Neuro-protectors"(Delivoria *et al.*, 1998). However, in adults, neuronal necrosis and apoptosis after global ischemia are slow, and last for several hours to several days (Ramaswamy *et al*., 2009). Studies in perinatal animals suggest a quicker cellular destruction. It is not known how long the window of opportunity remains open for intervention, but any intervention will be more successful early after the insult (Levene *et al.*, 1999). Energy substances in the neonatal brain continue to run down for 12 to48 h after hypoxia (Lorek *et al*., 1994). Therefore an intervention might be effective 2 to 6 h after birth asphyxia. As Neuro-protective interventions may be harmful. Levene *et al*. (1990) it is important to find early and reliable indicators of brain damage or of poor long-term prognosis to initiate or end Neuro-protective treatment: Cranial tomography, somatosensory evoked potentials, and magnetic resonance tomography are useful for prognosis, but not in the first 24 h after birth (Fitzhardinge *et al*., 1998) and (De Vries *et al*., 1991). Magnetic resonance spectroscopv reveals brain energy compromise, but is not practicable in most clinical situations. EEG is a useful diagnostic tool for assessing encephalopathy.

Previous reports Hellstrom-Westas *et al*. (l995) and Al –Naqeeb (1999) established a high predictive value of- postnatal EEG for neurologic outcome. Several studies measured biochemical factors in serum and cerebrospinal fluid glial fibrillary acidic protein after 12-48 h (Blennow *et al*., 1995), excitatory amino acids after 18-66 h (Hagberg *et al*., 1993) and InterLeukin-6(IL-6) 12 h after the hypoxic-ischemic event (Martin-Ancel *et al*., 1997). Urinary lactate: creatine ratio predicts HIE within 6 h with H nuclear magnetic resonance spectroscopy (Huang *et al*., 1999), 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 (Perlman and Teck, 1988). Our results do not confirm serum NSE as an early predictor of HIE. As late as 12 h after birth, serum concentrations of NSE increased significantly in the infants with moderate and severe" HIE when compared with infants with no or mild HIE. Results of other studies (Garcia-Alix, 1994) and (Thornberg, 1995) with serum NSE in asphyxiated newborns are in concordance with our data, whereas NSE in cerebrospinal fluid seems to be more favorable. Concerning CK-BB, these results are in accordance with studies where the increase was within the first 4-15 h of life (Cuestas, 1980 and Fernandez *et al*., 1987). Our serum CK-BB activities were lower than in the studies of Walsh *et al.* (1982) and Fernandez *et al*. (1987), which was probably related to the measurement of total creatinekinase activity at different temperatures. CK-BB levels in the neonate is negligible. 2S Cuestas (1980) found that the blood CK-BB levels were not increased in neonates with renal or gastrointestinal tract disorders. Therefore, we assume that increased levels originate mainly from the brain of the asphyxiated infants.

Protein S-100 is also known to be present in the striated muscle, heart kidneys, adipocytes, and thymus of newborns (Kojima *et al*., 1997). Until now, no study has examined the serum values of protein S-100 in asphyxiated and age-related control infants. In adults, protein S-100 is not detectable in serum under normal conditions (Fassbender, 1997) however, in cerebral diseases it ranged from zero (Buttaer, 1999) to highly predictive values (Martens *et al*., 1998). Additionally, clinical studies in adults evaluated different time patterns for the increase of (Marjaana *et al*., 2003) serum protein S-100, such as transient increase after cardiac operations (Westab *et al*., 1996) peak level within 24 h after cerebral hemorrhage (Kim *et al*., 1996) or global cerebral ischemia (Martens *et al*., 1998) and a peak level at d 3 after an acute ischemic stroke (Kim *et al*., 1996) and (Missler *et al*., 1997). In the serum of infants, protein V S-100 has been determined after cardiac operations and extracorporeal circulation, demonstrating age-related concentrations that were highest in neonates and infants with Down's syndrome with a pattern of transient increase similar to adult patients (Lindberg *et al.,* 1998). In asphyxia, serum protein S-100 release follows a pattern similar to the transient increase observed after cardiac operations. Leakage of protein S-100 into the extra-cellular fluid after hypoxic damage of blood-brain barrier seems to influence its level in serum, which explains why serum protein S-100 concentration is lower in adults than in infants (Lindberg *et al.,* 1998). Recent studies detected transient serum elevations of protein S-100 without any relationship to permanent neuronal damage, and the question has arisen as to whether protein S-100 arises from non-cerebral sources (Westaby *et al*., 2000 and Wirds *et al.*, 2003).

We could not exclude the possibility that protein S-100 and CK-BB release comes only from the brain. In regard to this, it might be a simple epiphenomenon 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. In our opinion, no single diagnostic marker should form the basis for decisions on Neuro-protective therapy. But the decision as to which infants could be candidates for post-asphyxial treatment should probably be based on several findings, which include EEC, Cord blood pH, Cord blood base deficit, Apgar score, serum protein S-100 and CK-BB. These biochemical markers may be helpful in deciding whether an early initiated Neuro-protective therapy should be continued or stopped. However, the obtained data from a small number of infants, refer to HIE and not permanent brain damage.

Conclusion:

It could be concluded that elevated serum concentrations of CK- BB and protein S-100 reliably indicate moderate and severe HIE as early as 2 h after birth (Good- win *et al*., 1992).

Recommendation:

Neuro- deveiopment follow-up studies in HIE infants in the next few years will show whether elevated serum protein S-l00 and CK-BB will also predict developmental delay.

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