ORIGINAL ARTICLE

Biochemical predictors of death before discharge in cooled newborns following perinatal asphyxia

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ABSTRACT

Aim To analyse biochemical markers as possible predictors of death before discharge in cooled newborns following perinatal asphyxia.

Methods A total of 91 infants that underwent therapeutic hypothermia after perinatal asphyxia were included. Inclusion criteria for therapeutic hypothermia were Sarnat stage 2 or 3. Data were collected from medical histories regarding gender, gestational age, birth weight, Apgar and Sarnat score; additionally, gas analyses, liver and cardiac enzymes before, and in the first 12 hours after starting therapeutic hypothermia, were evaluated. The patients' characteristics were compared between two groups, survivors and non-survivors.

Results Statistical difference was not found between groups regarding gender, gestational age, birth weight, delivery type, 1st and 5th minute Apgar score, seizures, alanine aminotransferase (ALT), creatine kinase (CK), troponin and fibrinogen level. Groups were significantly different regarding acid-base balance (p=0.012), base excess (BE) (p=0.025), lactate (p=0.002), aspartate aminotransferaze (AST), (p=0.011), lactate dehydrogenase (LDH) (p=0.006), activated partial thromboplastin clotting time (aPTT) (p=0.001) and international normalized ratio (INR) (p=0.001).

Conclusion Acid-base balance, BE, lactate, AST, LDH, aPTT and INR were significantly higher in the group of cooled newborns after perinatal asphyxia (non-survivors), and can serve as predictors of death before discharge. Combining diagnostic modalities raises a chance for accurate prediction of outcomes of asphyxiated infants.

Key words: hypoxia, biochemistry, treatment

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INTRODUCTION

Perinatal asphyxia is a significant cause of perinatal morbidity and mortality as well as neurological disabilities in survivors (1,2). It has been defined as lack of oxygen in perinatal period (3). A lot of efforts have been made in previous decades in order to prevent, recognize early and treat birth asphyxia. Still, neonatal encephalopathy due to perinatal asphyxia occurs in 1-3/1000 live births in high-income countries, and in up to 20/1000 live births in low and middle-income countries (such as Bosnia and Herzegovina, B&H) (4). Of affected newborns, 15-20% die in the neonatal period, and up to 25% who survive suffer from neurologic deficits (5). Perinatal asphyxia occurs due to maternal or placental events, intrapartum infection, or other different foetal conditions. Well known criteria for defining perinatal asphyxia are: metabolic acidosis with pH <7.0 in the first hour after delivery, base deficit ≥16, APGAR score ≤5 at 10 minutes, presence of multiple organ-system failures, clinical evidence of encephalopathy (1,2).

Hypoxic-ischemic encephalopathy (HIE) is defined as acute or subacute brain injury due to asphyxia. Clinical stages of HIE are given by Sarnat scale (2). The appropriate postnatal management of perinatal asphyxia includes: initial resuscitation and stabilization, adequate ventilation, perfusion and blood pressure management, as well as maintaining internal milieu in a referent range (glycaemia, minerals, etc.), treatment of seizures and therapeutic hypothermia for 72h (3-5).

Therapeutic hypothermia is considered to be the standard approach for treating neonatal HIE, and it is proven that it is the only certain neuroprotective therapy for infants with HIE (6,7). Lowering core body temperature for 1 °C results in a 6-10% reduction in whole-body metabolic demands (8). As many infants still suffer from perinatal asphyxia and go through the process of therapeutic hypothermia, there is a need to identify neonates who are at high risk for early neonatal death or severe neurological sequelae. In situation of limited resources, it is helpful to have simple and available method.

At our Clinic we do the whole-body cooling with the help of servo-controlled system (Arctic sun). Therapeutic hypothermia lasts for 72 hours. In case of lower resources, cooling can be done without servo-controlled systems, but with cooling devices including water-circulating cooling caps, frozen gel packs, ice, water bottles. In this case, the temperature is measured manually, with a thermometer, but this method is not reliable and the large temperature fluctuations can occur. During hypothermia, patients are under analgesia and sedated, on mechanical ventilation and parenteral nutrition and if necessary, anticonvulsants and inotropes are included in therapy.

The aim of this study was to analyse biochemical markers as possible predictors of death before discharge in cooled newborns following perinatal asphyxia.

PATIENTS AND METHODS

Patients and study design

This study was conducted at the Neonatal Intensive Care Unit (NICU), Paediatric Clinic, Clinical Centre of the University of Sarajevo during the period January 2016 to December 2018. A total of 91 infants after perinatal asphyxia who had undergone therapeutic hypothermia were included. Inclusion criteria for therapeutic hypothermia were Sarnat stage 2 or 3, minimal gestational age of 36 weeks, minimal birth weight of 2500 g, maximal postnatal age 6 hours, Apgar score 10 minutes after birth ≤5, or need for resuscitation 10 minutes after birth, or pH <7.00, or base excess (BE) ≥-16mmol/L within 60 minutes after birth.

Methods

Therapeutic hypothermia was provided via servo control total body cooling. Seventy-two-hour therapeutic hypothermia started within 6 hours after birth. A target temperature was 33.5 °C. During whole body cooling patients were mechanically ventilated, with analgosedation, magnesium to maintain level 1.0 mmol/L, anticonvulsive drugs, inotropic agents if needed, and broad spectrum antibiotics. Parenteral intake was limited to 60 mL/kg/day. Urine output and all vital parameters were continuously monitored.

The patient's data were collected from medical histories: gestational age, birth weight, Apgar and Sarnat score, gas analyses, liver and cardiac enzymes before and in the first 12 hours after starting therapeutic hypothermia. Patients' cha-

racteristics were compared between two groups, survivors and non-survivors.

Statistical analysis

Quantitative variables were evaluated regarding mean value and standard deviation. While comparing survivors with children who died, Student t test and $\chi 2$ test were used. Statistical tests were carried out at the 5% significance level.

RESULTS

Inclusion criteria were fulfilled by 91 patients, of which 53 (58.3%) were males (p=0.494). Mean body weight of all infants was 3404.8±717.04 g (2500-5175g). Mean gestational age was 39.15±1.88 (36-42). More than one third of patients, 36 (39.56%), were graded as severe asphyxia (Sarnat 3). One third of the patients were inborns, 31 (34.06%), and the rest were transferred from lower-level hospitals and deliveries (outborn).

Mean Apgar in the 1st minute was 2.53±1.66 (0-4), and in the 5th minute 4.12±1.65 (0-7). Mean pH was 6.99±0.18, mean EB -18.29±6.9. Mean lactate level in the first 6 hours was 12.33±5.55 (7.9-22). Mean values of biochemical parameters were: AST 243.15, ALT 98.08, LDH 1376.02, APTT 65.46, INR 2.38, troponin 0.23 (Table 1).

Table 1. Characteristics of newborn patients according to two groups

Variable	Mean (range)		p
	Survivors	Non-survivors	
Gestational age (weeks)	39.05 (38-40)	38.09 (37-41)	0.926
Birth weight (grams)	3484.52 (3100-3690)	3399.44 (2870-3910)	0.865
1st min Apgar	2.61 (0-4)	2 (1-3)	0.193
5th min Apgar	4.28 (2-8)	3.4 (1-7)	0.071
Seizures			0.711
Yes	37	10	
No	36	8	

As eighteen (19.78%) patients died before discharge (six were inborn, while the others were outborn), we created two study groups: survivors and non-survivors. No statistical difference was found between the groups regarding gender, gestational age, birth weight, delivery type, 1st and 5th minute Apgar score, seizures, ALT, CK, troponin and fibrinogen level. The groups were significantly different regarding following parameters: pH, BE, lactate, AST, LDH, APTT, and INR (Table 2).

Table 2. Values of biochemical parameters of newborn patients according to two groups

Variable	Mean (range)		p
	Survivors	Non-survivors	
Acid base status (pH)	7.05 (6.9-7.13)	6.92 (6.67-7.05)	0.012
Base excess	-17.0 (-22--13)	-22.1 (-25.8--19)	0.025
Lactate (mmol/L)	10.60 (7.9-14.4)	18.0 (12.5-22)	0.002
Aspartat aminotransferase (U/L)	133 (81-210)	297 (124-498)	0.011
Alanine aminotransferase (U/L)	46 (27-81)	108 (23-125)	0.162
Creatine kinase (U/L)	1304 (724-2705)	1690 (862-2141)	0.704
Lactate dehydrogenase (U/L)	903 (636-1280)	1629 (1263-2236)	0.006
International normalized ratio	1.85 (1.55-2.61)	3.02 (2.01-4.03)	0.001
Activated partial thromboplastin clotting time (seconds)	52.6 (45.1-67)	78.4 (62.4-99)	0.001
Troponin (ng/mL)	0.1150 (0.047-0.219)	0.149 (0.091-0.389)	0.125
Fibrinogen (mg/dL)	1.9 (1.3-2.3)	1.8 (1.6-2.1)	0.874

We found 51.6% patients had seizures (clinically detected, or aEEG confirmed) without difference between survivors and the dead (p=0.71). Six of 31 inborn patients died, and 12 of 60 outborn patients. The relative risk (RR) for death of outborn patients was 1.05.

DISCUSSION

In this study we found no difference regarding gestational age, birth weight, gender, and Apgar scores in the 1st and the 5th minute between survivors and non-survivors. The higher prevalence of birth asphyxia in males was noticed by several authors (9-11) including this study. Death rate reported in our study was 19.78%, which is in accordance with other studies, ranging from 3.2% (12) to 28% (13).

Our results showed that two thirds of patients were transferred from other units (outborn). Data from the Vermont Oxford Encephalopathy Registry indicated >60% outborn babies, and data from Chang Gung Memorial Hospital demonstrated that three-fourths of enrolled neonates were outborn (14). This dominance of outborn patients with HIE should alert us to improve our efforts considering prenatal care, labour, newborn resuscitation, and transport (15). As in neonates with perinatal asphyxia hyperoxemia increases the risk of abnormal brain magnetic re-

sonance imaging findings, careful use of oxygen during resuscitation is necessary (16). In respect to this fact and according to the 2019 American Heart Association Focused Update on Neonatal Resuscitation (17), we use a low dose of oxygen (room air for term infants and 21-30% oxygen for preterm as a starting value) during the resuscitation process. Hypoxic-ischemic encephalopathy is the most common cause of seizures in newborns, and there is a well-known connection between neonatal seizures and morbidity and mortality (18). Animal studies indicate that therapeutic hypothermia due to HIE can reduce seizures (19-21). In spite of reports of lower incidence of seizures in cooled infants (21), some studies have failed to show an association between therapeutic hypothermia and reduced seizure burden, and only one meta-analysis has reported the predictive characteristics of amplitude-integrated electroencephalography (aEEG) regarding therapeutic hypothermia (22-24). Other studies reported prevalence of seizures in cooled infants form 26% (moderate HIE) to 87% (severe encephalopathy) (18).

Many previous studies noticed that serum liver enzyme elevation is common in birth asphyxia. Chhavi et al. found that this elevation peaks at 24-72 hours after birth, followed by a sharp decline by 6-12 days of age (25).

A weak point of this study is that we did not take into consideration AST and ALT levels in correlation with stage of asphyxia like a study from 2016 that showed in case of ALT the difference of the mean values significant in Stages 1 and 2 and Stages 1 and 3, but insignificant in Stages 2 and 3 (11). However, Tariqul et al. concluded that only the specificity of ALT can be used as an indicator of hepatic involvement in perinatal asphyxia with AST being non-significant (26). The same authors (11) found a better diagnostic value of LDH than other liver enzymes in predicting perinatal asphyxia (LDH had 100% sensitivity and 99% specificity at cut-off value of 1633), similarly to our results.

A study by Nakajima et al. took into consideration a correlation between the serum-leaked intracellular enzyme levels at the beginning of the treatment and the outcome in perinatally stressed neonates (27). They showed significantly larger base deficit, and higher lactate, AST, ALT, LDH, and CK in the therapeutic hypothermia group, and the duration of mechanical ventilation significantly correlated with AST, ALT, LDH, and CK levels. Abnormalities of coagulation occur when there is substantial impairment in the ability of the liver to synthesize these factors, so it can serve as a measure of liver dysfunction (28). As a study from 2015 concluded prothrombin time and INR were significantly higher in asphyxiated neonates, and hypoproteinemia, prolonged prothrombin time and INR were noted to be the ominous signs predicting mortality (26). In our study INR and APTT were significantly higher in non-survival group. We did not find a difference in fibrinogen levels, but Sweetman et al. have found APTT levels increased while fibrinogen and platelet levels significantly decreased in infants who required TH, had grade II/III of neonatal encephalopathy and in non-survivors (29). In this study we found lactate level significantly higher in non-survivors, being a possible predictive factor of death between asphyxiated infants. An older study also found the initial lactate levels significantly higher ($p=0.001$) in neonates with moderate-to-severe HIE as compared to those with mild or no HIE, and lactate levels took longer to normalize in these babies (30). The sensitivity and negative predictive value of lactate was greater than that of the pH or base deficit (31). In our study pH in the first hours of life was significantly higher in non-survivors group.

Contrary to an Indian study showing troponin-T concentration to have a good predictive accuracy for mortality before discharge (32), we found no statistically significant difference between the groups. Studies showed that aEEG at 36 h, predictors of congenital anomalies on magnetic resonance imaging (MRI), apparent diffusion coefficient (ADC) values of the thalamus and magnetic resonance spectroscopy (MRS) are the most predictive of adverse (neurodevelopmental) outcome. The EEG at 24 h and 72 h after birth were superior to aEEG at 6 h and EEG at 48 h after birth (32-34). Early conventional MRI in the first week of life was preferred over late MRI.

In conclusion, this study showed that pH, BE, lactate, AST, LDH, APTT, INR are significantly higher in the group of cooled newborns after perinatal asphyxia, who died before discharge. Those biochemical markers can serve as predictors

of death before discharge in cooled newborns following perinatal asphyxia. Combining diagnostic modalities raises a chance for accurate prediction of outcomes of asphyxiated infants.

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