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RESEARCH ARTICLE

High antioxidant capacity may alter the severity and treatment of hypoxic-ischemic encephalopathy in newborns

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ABSTRACT

Background: We compared prooxidant and antioxidant capacity in newborns with mild, moderate, and severe hypoxic-ischemic encephalopathy with a control group.

Methods: Twenty-eight newborns were included in the perinatal asphyxia group and 32 healthy newborns were included in the control group. Newborns in the perinatal asphyxia group were divided into two groups as mild hypoxic-ischemic encephalopathy (n=12) and moderate+severe hypoxic-ischemic encephalopathy (n=16) groups according to the Sarnat Classification. Basal venous blood samples were taken from the newborns in the mild hypoxic-ischemic encephalopathy (n=12), moderate+severe hypoxic-ischemic encephalopathy (n=16) and the control group (n=32) at the postnatal 1st hour, and the level of total oxidative stress and total antioxidant capacity were studied. Oxidative stress index was calculated as the percent ratio of total oxidative stress to antioxidant capacity in all newborns.

Results: Total antioxidant capacity level was significantly higher in the mild hypoxic-ischemic encephalopathy group (n=12) compared to the control group (n=32) (p=0.04). No statistically significant difference was found between the mild hypoxic-ischemic encephalopathy, moderate+severe hypoxic-ischemic encephalopathy and control groups in terms of total oxidative stress and oxidative stress index

Conclusion: High antioxidant capacity predicted the diagnosis of mild hypoxic-ischemic encephalopathy. There is a need for studies showing a cut-off value for antioxidant capacity as a biomarker in the diagnosis of HIE. Maternally given antioxidant therapy can be neuroprotective in newborns with hypoxic-ischemic encephalopathy where there is a clinical picture of mild hypoxic-ischemic encephalopathy in newborns with a high antioxidant capacity. Further studies are needed to evaluate whether maternal antioxidants are neuroprotective in newborns.

Keywords: hypoxic ischemic encephalopathy, antioxidant capacity, maternal antioxidant therapy, newborn

Introduction

Hypoxic ischemic encephalopathy (HIE) contribute significantly to neonatal morbidity and mortality in term newborns despite therapeutic hypothermia treatment [1]. Hypoxic ischemic encephalopathy incidence is estimated as 1.5 per 1000 live births (95%CI 1.3 to 1.7). Its incidence is 8/1000 in developed countries and 25/1000 in developing countries [2]. In the pathogenesis of HIE, reactive oxygen species (ROS) increasing with hypoperfusion and reperfusion [superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), peroxynitrite $ONOO^-$)] have a key role in brain injury. Reactive oxygen species induces inflammation, apoptosis, autophagy and necrosis by directly degenerating or modifying cellular macromolecules such as membranes, proteins, lipids and DNA [3]. The clearance of ROS is provided by superoxide dismutase, catalase, glutathione and glutathione peroxidase that are antioxidant enzymes [4 – 5]. Newborn brain is susceptible to hypoxia due to high-concentration of sensitive immature cells, unsaturated fatty acids and a high oxygen demand as well as high levels of free radicals, low levels of antioxidant enzymes and high oxidative stress [6]. Although newborns are known to be susceptible to asphyxia, it is still unknown why they are not affected in the same way. The degree of asphyxia is classified by Sarnat [7] into mild, moderate, and severe hypoxic-ischemic encephalopathy. Hyperalert consciousness, normal muscle tone, normal posture, hyperactive deep tendon reflexes (DTRs), presence of myoclonus, strong Moro reflex, mydriatic pupils, no seizures in Mild HIE. Moderate HIE is defined as lethargic consciousness, hypotonic tonus, flexion posture, hyperactive DTRs, presence of myoclonus, poor moro reflex, miotic pupils, presence of seizures. Severe HIE is defined as coma state, flaccid tonus, decerebrate posture, undetectable DTRs, absence of myoclonus, undetectable moro reflex, anisocoric pupils. Besides newborn with mild HIE that have no indication for treatment, there are newborns with moderate-severe HIE who have a poor prognosis despite hypothermia treatment. In this study, we aimed to evaluate whether oxidative damage affects the clinical findings of HIE by using the Erel method.In this study, blood levels of total oxidative stress (TOS) and total antioxidant capacity (TAC), and oxidative stress index (OSI; TOS/TAC) were investigated in mild HIE (no hypothermia) and moderate+severe HIE (hypothermia) groups of newborns with perinatal asphyxia and control group in order to answer the question that do postnatal prooxidant and antioxidant capacities predict the clinical severity of hypoxic ischemic encephalopathy?

Objective

To compare prooxidant (TOS, OSI) and antioxidant capacity (TAC) in newborns with mild(no hypothermia) HIE, moderate + severe HIE(hypothermia) and control groups.

Methods

PATİENTS AND STUDY DESİGN

Newborns with the diagnosis of perinatal asphyxia[8] who were admitted to the neonatal intensive care unit of the Zeynep Kamil Maternity and Children's Research and Training Hospital (n=28) and healthy newborns who were born at Zeynep Kamil Maternity and Children's Research and Training Hospital (n=32) between January 2016 and December 2016 were included in the study. The sample size in the study was reached by using the open epi program by taking the incidence of HİE as 1.5 per 1000 live births.

Newborns diagnosed with perinatal asphyxia (n=28) were divided into two groups as mild HIE (no hypothermia) (n=12) and moderate+severe HIE (hypothermia) (n=16) according to the Sarnat Classification[7] (level of conciousness, muscle tone, posture, tendon reflexes, myoclonus, pupillary reaction, seizure and aEEG).

Venous blood samples were collected from the newborns in the mild HIE, moderate+severe HIE and control groups at the postnatal first hour, and prooxidant capacity [total oxidative stress (TOS)], and antioxidant capacity [total antioxidant capacity (TAC)] were studied. Oxidative stress index (OSI) was calculated as the percent ratio of TOS to TAC in all newborns.

Definition

PERİNATAL ASPHYXİA [8]

The diagnosis of perinatal asphyxia was established in patients who have one or more symptoms according to the following American College of Obstetricians and Gynecologists' criteria:

Regarding acute peripartum or intrapartum status: having an apgar score < 5 at the 5th and 10th minutes; cord pH < 7 or base deficit <-12 mmol/l; development of multiorgan failure. **Presence of a condition contributing to acute peripartum or intrapartum status in the infant:** presence of a hypoxic or ischemic event during or a short time before delivery; ruptured uterus; severe abruptio placenta; prolapse of the umbilical cord; hypoxemia; prolonged maternal hypotension accompanied with amniotic fluidembolism; maternal cardiovascular collapse; massive fetomaternal hemorrhage or fetal blood loss with vasa previa; if fetal heart beat pattern converts into to category 3 from category 1, or to tachycardia with recurrent deceleration, or to persistent minimal viability with recurrent deceleration during acute peripartum or intrapartum events; absence of these conditions: abnormal fetal growth, maternal infections, fetomaternal bleeding, neonatal sepsis, chronic placental lesions

SEİZURE, BURST SUPPRESSİON, CONTİNUOUS VERY LOW VOLTAGE, İSOELECTRİC LİNE İN AEEG

Seizure was defined as sudden elevation in aEEG activity band upon narrowing during active body cooling. A discontinuous ground on aEEG with a lower amplitude of 0-1 (2) µV and bursts > 25 µV was defined as **burst suppression**, an activity with an amplitude about 5 µV or lower was defined as **continuous very low voltage**, and an inactive ground under 5 µV was defined as **isoelectric line.**

Pathological findings on ECHO were defined as decreased biventricular output, increased biventricular myocardial performance index and global systolic and diastolic dysfunction and pulmonary hypertension [9].

BLOOD SAMPLİNG AND BİOCHEMİCAL STUDİES

Blood concentrations of TAC (mmol trolox equiv/L), TOS (mmol H2O2 equiv/L). Blood samples were centrifuged for 3 minutes at 5000 rpm, and plasma samples were stored at -80 C◦. Serum TAC and TOS levels were measured, as described by Erel[10 11] . Erel's method for serum TAC level measurement is based on the bleaching of the characteristic color of a more stable 2,2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation by antioxidants.

The results were expressed in mmol trolox equivalents/L. Erel's TOS measurement is based on the oxidation of ferrous ions to ferric ions in the presence of various oxidative species and the measurement of the ferric ion by xylenol orange. The results were expressed in mmol H2O2 equivalents/L. Oxidative stress index [arbitrary unit = TOS (mmol H2O2 equiv/L) / TAC (mmol trolox equiv/L)] was calculated as the percent ratio of TOS to TAC.

Statistical analysis

During the assessment of the data obtained in the study, SPSS version 15 (SPSS Inc., Chicago, IL, USA) program was used for statistical analysis. Median values of nonparametric tests were reported with minimum and maximum values. Kruskal-Wallis test was used to evaluate the significance of the differences between the mean values of three or more non-normally distributed groups. Significant values in the Kruskal-Wallis test ($p<0.05$) were compared with Mann-Whitney U test. Non-normally distributed numerical and ordinal variables were compared using the Mann Whitney U test. Statistical significance level was set at $p<0.017$ in the Mann-Whitney U test. Student t test was used for the comparison of parametric variables. Chi-square test was used to compare the categorical variables. Analysis of the variance test was used to compare mean values among more than two groups.

Results

A total of 13076 term infants were admitted to the neonatal intensive care unit of the Zeynep Kamil Maternity and Children Diseases Training and Research Hospital within 1 year. The incidence of perinatal asphyxia was 28 (0,2%) in term infants. Demographic data and apgar scores were evaluated in mild HIE (n=12), moderate+severe HIE (n=16) and control (n=32) groups. No significant difference was found among all three groups in terms of gestational week, birth weight, maternal age and cesarean section. There was a significant difference between all three groups in terms of the 1st and 5th minutes apgar scores ($p <0.001$, $p<0.01$; respectively). When evaluated with the Mann-Whitney U test; 1st and 5th minutes apgar score was significantly lower in the mild HIE and moderate+severe HIE groups compared to the control group ($p<0.001$, $p<0.001$; $p<0.001$, $p<0.001$; respectively), while 5th min apgar score was significantly higher in the mild HIE groups compared to the moderate + severe HIE group ($p= 0.004$) (Table 1).

Table 1: Demographic data and Apgar scores of the newborns in the mild HIE, Moderate + severe HIE and control groups

	Mild HIE n=12	**Moderate+severe HIE n=16**	**Control n=32**	**p**
Gestational week	39.5 (37-42)	38 (36-42)	39 (37-41)	0.394
Birth weight, g	3295 (2415-4000)	3107 (2080-4200)	3740 (2600-4600)	0.138
Maternal age	28.5 (18-38)	28 (18-38)	27.5 (20-38)	0.779
C/S, n (%)	2(16)	8(50)	9(28)	0.171
1th min Apgar (min-max)	4 (0-7)	2.5 (0-5)	7 (6-8)	p 1= 0.02 p 2<0.001 p3<0.001
5th min Apgar (min-max)	8 (5-9)	5 (3-8)	9 (8-9)	p 1=0.004 p 2<0.001 p 3<0.001

Comparison of the significant findings in the Kruskal-Wallis test with Mann-Whitney U test ($p<0.017$: significant) p 1: Mild HIE / Moderate + Severe HIE; p 2: Mild HIE / Control; p3: Moderate + Severe HIE / Control

The incidence of seizures, low voltage, and burst suppression on aEEG was higher in the moderate + severe HIE group than in the mild HIE group ($p=0.004$, $p=0.03$, $p=0.004$;respectively). Postnatal 1st hour blood gases and enzymes were compared between the patients in the mild HIE and moderate+severe HIE groups. Blood pH and HCO3 werelower ($p<0.001$, $p<0.001$; respectively), and uric acid and BE levels were higher in the moderate+severe HIE group than in the mild HIE group ($p<0.048$, $p<0.001$; respectively). No significant difference was found between the two groups in terms of CO_2, Lactate, CK, CK- MB, LDH and troponin (Table 2).

Table 2: Pathologic aEEG, ECHO, BERA evaluations, blood gas and laboratory findings of the newborns in the mild HIE and Moderate + severe HIE groups

	Mild HIE n=12	**Moderate+severe HIE n=16**	**p**
aEEG-epileptic focus (%)	0	8 (50)	0.004
aEEG- low voltage (%)	2(16)	9(56)	0.003
aEEG-burst (%)	0	8(50)	0.004
ECHO-TY, AY(%)	3 (25)	7 (43.8)	0.345
Ph	7.22 (7-7.4)	6.9 (6.6-7.1)	<0.001
PCO2 (mmhg)	51 (22-101)	80 (28-107)	0.082
HCO3(mmol/l)	17 (11-22)	10.5 (4-10)	<0.001
BE (mmol/l)	- 8 (-18- -7)	-18 (-33- -11)	<0.001
LACTATE (mmol/l)	9 (3-19)	12.2 (5-18)	0.156
CK (U/L)	1138 (189-2449)	1156 (504-14302)	0944
CK-MB (U/L)	23.6 (4.9-152)	27.2 (8-366)	0.384
LDH (U/L)	1066 (626-2745)	1348 (623-18621)	0.521
TROPONIN (ng/ml)	0.04 (0.01-1.18)	0.03 (0.01-0.19)	0.778
URIC ACID (mg/dl)	7.2 (3.2-9.7)	8.3 (5.9-12.8)	0.048

When the levels of blood TAC and TOS levels and OSI ratio were compared between the groups; TAC was found to show significant difference between all three groups (p=0048). Mann-Whitney test was applied to determine the group that created the difference and TAC was found to be higher in the mild HIE group compared to the control group (p=0.016). There was no statistically significant difference between the mild HIE and moderate+severe HIE groups (p= 0.051) and between the moderate+severe and control groups (p= 0.728) in terms of TAC. There was no statistically significant difference between the three groups compared to the blood level of TOS and OSI (Table 3).

Table 3: TAC, TOS and OSI values of the newborns in the mild HIE, Moderate + severe HIE and control groups

	Mild HIE n=12	**Moderate+Severe HIE n=16**	**Control n=32**	**p**
TAC*(mmoltrolox equiv/L)	2.38 (1.6-2.86)	2.03 (0.85-2.84)	2.1 (1.6-2.2)	0.048
TOS**(mmolH2O2 equiv/L)	23.8 (16.2-85.1)	35.1 (13.1-59.8)	23.5 (17-44)	0.258
OSI***	9.97 (5.8-53.5)	17.3 (5.8-70.4)	12 (8.5-20)	0.159

* Total antioxidant capacity, **Total oxidative stres, *** Oxidative stress index

Discussion

In our study, we found a high antioxidant capacity in mild HIE. High antioxidant capacity predicted the diagnosis of mild HIE. There are numerous studies about prooxidant biomarkers in the diagnosis of HIE. In all these studies, high prooxidant marker levels have been found in severe HIE [12–15] . In our study, no significant correlation was found between prooxidant capacity and clinical picture of HIE. The number of studies with antioxidants on this issue are limited. A positive correlation has been found between the clinical severity of HIE and cord superoxide dismutase and catalase and blood level of superoxide dismutase at 24 hours [16] . In our study, a mild HIE clinical picture was found in the newborns with a high antioxidant capacity. This result, which is contrary to the previous study, may be due to the fact that the antioxidant status was evaluated with a different method in our study. There is a need for studies that will investigate cut-off value for antioxidant capacity in the diagnosis of HIE. As shown in our study, maternally given antioxidant therapy can be neuroprotective in newborns with HIE if the clinical picture of mild HIE develops in newborns with a high antioxidant capacity. In limited studies, in which two antioxidants, melatonin and allopurinol were maternally administered, the effectiveness of these antioxidants in the treatment of HIE is controversial. In a study by Miller et al. in 2005, melatonin given to maternal sheep was shown to reduce the production of free oxygen radicals in the fetus and to have neuroprotective effect [17]. In a study by Taurence et al. in 2009, maternally administered allopurinol was demonstrated to pass the placenta throughout fetal hypoxia and decrease cord blood level of S-100B that is a brain injury marker [18] . In the multicenter, randomized ALLO study by Kaandorp et al. in 2015, no decrease could be shown in cord lipid peroxidation and neuronal injury markers in asphyxic fetus with maternally

administered allopurinol [19]. When long-term neurological effects of the ALLO study were evaluated in 2018; no effect of allopurinol on 5-year developmental behavioral improvement could be demonstrated [20]. In the first two studies, maternally given antioxidants were found to be neuroprotective, while in the other two studies, it was found that antioxidants were not neuroprotective. In the last two studies, short and long term results of the same study were evaluated and, no significant difference was found between the newborns in the maternal control group and allopurinol administered group in terms of apgar score, pH and base deficit, and it is noteworthy that there were no HIE in both groups. Liu and colleagues study has highlighted treatments targeting the transcription of antioxidant genes in hypoxic-ischemic encephalopathy [21].

Conclusion

Our study has shown that infants born with high antioxidant capacity develop mild hypoxic-ischemic encephalopathy. Therapeutic hypothermia is not indicated for patients with mild ischemic encephalopathy. Their neurological development is good. By administering maternal antioxidants, the severity of hypoxia in asphyxiated infants born with high antioxidant capacity can be reduced, and neurological outcomes can be improved. Further studies are needed in this area.

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