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Evaluation of Plasma Level of Heme-Oxygenase-1 in Neonatal Hypoxic Ischemic Encephalopathy

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ABSTRACT

Background: Neonatal hypoxic-ischemic encephalopathy (HIE) is one of the most common causes of long-term neurological disabilities among children. Various types of cellular stress stimuli, including oxidative stress, inflammation, and hypoxia, induce heme oxygenase-1 (HO-1) enzyme for different kinds of tissues. The purpose of this study was to evaluate the plasma level of HO-1 enzyme in neonatal HIE patients and to determine the relationship between HO-1 enzyme level and clinical severity of HIE.

Methods: In this case-control study, the plasma level of HO-1 enzyme was measured through sandwich ELISA in 28 newborns with a proven diagnosis of HIE and 31 healthy full-term newborns admitted to Bentolhoda Hospital, Bojnourd, Iran. Newborns with HIE were classified according to the Sarnat staging to mild, moderate, and severe HIE. Maternal and neonatal data were recorded in checklists and compared between the two groups.

Results: The mean plasma level of HO-1 enzyme in HIE patients was significantly higher than that in the control group (104.0 ± 4.01 and 91.63± 2.67 pg/ml, respectively, P=*0.011*). We also found that plasma HO-1 levels were significantly higher in severe neonatal HIE patients compared to mild and moderate neonatal HIE patients (121.0 ± 8.48Vs. 91.23 ± 3.35 and 105.5 ± 5.76, $P < 0.001$).

Conclusion: Our findings suggested that HO-1enzyme may be associated with the pathophysiology and clinical severity of neonatal HIE. We suggest further research on the correlation of plasma level of HO-1 enzyme at birth with the multi-organ dysfunction and abnormal neurodevelopmental outcomes in full-term newborns with HIE.

Keywords: Hypoxic ischemic encephalopathy, Heme oxygenase-1, Newborn, Plasma

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Introduction

Neonatal hypoxic-ischemic encephalopathy (HIE) is among the most common causes of long-term neurological disabilities among children, particularly in the newborns (1,2). The incidence rate of that perinatal asphyxia is 1.5 per 1000 live births in developed countries and varies between 2.3-26.5 per 1000 live births in developing countries (3,4). Our center has already shown that perinatal asphyxia has been reduced considerably after implementing the health improvement program (0.54% versus 1.05%) (4).

Furthermore, the mortality rate of this event in neonatal intensive care units (NICU) has been reported to be 24% in the developing countries [5]. Among the remaining alive newborns, cerebral palsy (10–20%), visual and auditory problems (40%), as well as motor and behavioral damages, such as epilepsy, global developmental delay, and autism are diagnosed (5). Because the injury during HIE evolves for days and possibly weeks, clinicians must be aware of the pathophysiology of this insult to manage the affected newborns appropriately and provide strategies for early prediction, diagnosis, and treatment of HIE (6). The majority of the underlying pathologic events of HIE are a result of impaired cerebral blood flow and oxygen delivery to the brain with resulting primary and secondary energy failure (7-8). The impairment of cerebral blood flow in primary energy failure leads to decreases in oxygen and glucose, which leads to significantly less energy (ATP) and increased lactate production (7-8). The secondary energy failure phase occurs 6-48 hours after the initial injury and is related to oxidative stress, excitotoxicity and inflammation (7).

The plasma carbon monoxide (CO) level after perinatal asphyxia is related to the severity of neonatal HIE (9). CO is produced endogenously in humans as a by-product of heme degradation catalyzed by the heme oxygenase-1(HO-1) enzyme (10). HO-1 enzyme, responsible for heme degradation to biliverdin/bilirubin, free iron, and CO, has been heavily implicated in mammalian brain damage and CNS disease (10). Genetic experiments showed an essential role for endogenous HO-CO system in tissue homeostasis, protection against apoptosis, inflammation, oxidative stress, and in the pathogenesis of the disease (11, 12).

Although the HO-1 enzyme is related to the pathologic outcome of such a broad spectrum of disease conditions, minimal data have been reported about the HO-1 enzyme in animal and human models of HIE (13-15). The purpose of this study was to evaluate the plasma level of HO-1 enzyme in neonatal HIE patients and to determine the relationship between HO-1 enzyme level and clinical severity of neonatal HIE.

Materials and Methods

This case-control study was conducted on 28 full-term neonates diagnosed with neonatal HIE (case group) delivered at the Obstetrics and Gynecology Department and admitted to the Neonatal Intensive Care Unit (NICU) of Bentolhoda Hospital, Bojnourd, Iran, between September 2020 and December 2020. Neonatal HIE was diagnosed as described earlier by Martinello *et al*. (15) and Wang *et al.* (16)., through meeting the following three criteria: (a) any three features of encephalopathy within 72 hours of birth, such as abnormal level of consciousness (e.g., hyper-alert state, lethargy, stupor or coma), abnormal muscle tone, abnormal deep tendon reflexes, seizure, abnormal Moro reflex, abnormal sucking reflex, abnormal respiratory pattern, and oculomotor or pupillary abnormalities, (b) three or more findings of acute perinatal events, such as arterial cord pH < 7.00, Apgar score < 5 at five minutes of life, evidence of multi-organ system dysfunction within 72 hours of birth, evidence of fetal distress on antepartum monitoring, abnormal electroencephalogram, and abnormal imaging of the brain showing ischemia or edema within seven days of birth, and (c) the absence of any underlying congenital cerebral infections/abnormalities or inborn errors of metabolism that could account for the encephalopathy.

In the present study, neonates with HIE were classified into stage I (mild), stage II (moderate), and stage III (severe) according to the Sarnat and Sarnat staging system 17,18, which evaluates the level of consciousness, muscle tone, tendon reflexes, complex reflexes, and autonomic function.

In total, 31 healthy full-term newborns matched for age and sex were included in the study as the control group. A neonatologist collected and recorded the maternal and neonatal data, perinatal history, and clinical and laboratory findings of the subjects. Data collection was performed by reviewing the newborns' medical records, either diagnosed with perinatal asphyxia or healthy babies.

Parents of neonates who were participated in the study gave written informed consent after explaining the procedure and purpose of the study. The present study was conducted under the Declaration of Helsinki and with approval from the Ethics Committee of North Khorasan

University of Medical Sciences, Bojnurd, Iran (IR.NKUMS.REC.1396.373).

Newborns were excluded from the study if they met any of the following conditions: significant congenital anomaly, sepsis, congenital malformations, congenital or perinatal infections, metabolic disorders, hemolytic illness, hemorrhagic shock, maternal drug addiction, identified or suspected primary hepato-biliary disease and absence of parental consent.

Umbilical cord blood samples were obtained from newborns (case and control groups) in heparinized tubes at the time of birth. Blood samples were centrifuged for 10 minutes at 800× g, and plasma was separated and stored at -20°C until analysis.

The level of HO-1 enzyme in neonatal plasma was assessed according to the manufacturer's standard procedure by a quantitative sandwich enzyme-linked immunoassay (ELISA, Abcam, USA). Samples were tested in triplicate. The minimum detectable concentration was 6pg/ml.

Statistical Analysis

GraphPad Prism software version 5.0 (GraphPad Software, USA) was used for statistical analysis of the data. Data distribution was analyzed by Kolmogorov–Smirnov test. According to the normality test results, comparisons between the two groups were made using two-tailed Student's t-test and chi-squared test for continuous and parametric variables. Kruskal-Wallis test was used for data with the non-Gaussian distribution. Data is shown as the mean ± SEM of three independent experiments. *P* values < 0.05 were considered statistically significant.

Results

In total, 59 neonates were enrolled in this study and categorized into the case group (HIE neonates, n= 28) and the control group (healthy full-terms, n=31). The neonatal HIE patients were classified as mild (n=11, 39.28%), moderate (n=9, 32.14%), and severe (n=8, 28.57%) using Sarnat and Sarnat staging systems.

We found no significant difference between neonatal HIE patients and control group in terms of mean birth weight (2.75 ± 0.62 kg vs. 2.99 ± 0.50 kg), mean gestational age (37.72 ± 3.77 weeks vs. 39.21 ± 1.42 weeks), gender, and mode of delivery (P > 0.05). The Apgar scores at 1 and 5 minutes were significantly lower in the patients' group compared to the control group, as shown in Table 1 (P < 0.05). The arterial blood gas analysis results in neonatal HIE patients showed the following values: mean pH of 7.09 ± 0.19, PCO2 of 61.99 ± 4.36 mmHg, HCO^-_3 of 19.66 ± 1.79 mmHg, base excess (BE) of<– 12mmmol/L.

Table 1. Demographic and Clinical Data of Study Population (n=59)

Variables		Case *(*HIE) (n=31)	Control (n=28)	*P* value
Maternal Age (years)		28.03 ± 7.39	27.21 ± 6.56	0.65
Gravidity	Primigravida (n, %)	11 (35.5%)	18 (64.3%)	0.02
	Multigravida (n, %)	20 (64.5%)	10 (35.7%)	
History of Miscarriage	Positive (n, %)	5 (16%)	9 (32%)	0.15
	Negative (n, %)	26 (84%)	19 (68%)	
Gestational Age (weeks)		37.72 ± 3.77	39.21 ± 1.42	0.05
Weight (kg)		2.75 ± 0.62	2.99 ± 0.50	0.11
Gender	Male (n, %)	18 (58)	16 (57)	0.94
	Female (n, %)	13 (42)	12 (43)	
Apgar Score	1 minute	3.66 ± 1.39	9.00 ± 0.27	0.001
	5 minute	5.50 ± 1.38	9.96 ± 0.18	0.001
Mean pH of Cord Blood		7.09 ± 0.19	7.37 ± 0.01	0.001

Our results showed that the mean concentration of HO-1 in the control and HIE group were 91.63± 2.67 and 104.0 ± 4.01 pg/ml, respectively. As shown in Figure 1, the plasma level of HO-1 in neonatal HIE patients was significantly higher than that in the control group (P=0.011).

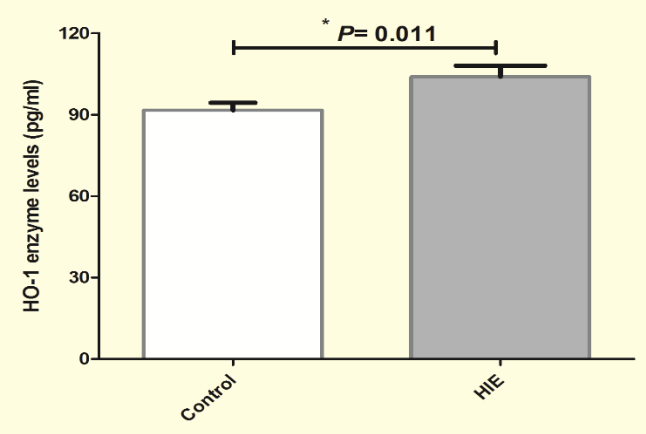

Figure 1. Plasma level of HO-1 in neonatal HIE and healthy full-term newborns (as control group).

Plasma level of HO-1 shows significant difference between the HIE group and control group when the *P* value is equal to 0.011. HIE: Hypoxic Ischemic Encephalopathy, HO-1: Heme-Oxygenase-1, Data represent mean ± SEM.

Figure 2 shows HO-1 level in three groups of neonatal HIE patients (mild, moderate, and severe) in comparison to the control group. As it is seen, plasma HO-1 level in sever and moderate neonatal HIE patients, but not in mild neonatal HIE patients, is significantly higher than the same value in the control group ($P < 0.05$). Further analysis showed significantly higher plasma HO-1 level in severe neonatal HIE patients compared to mild neonatal HIE patients (121.0 ± 8.48 pg/ml Vs. 91.23 ± 3.35 pg/ml, P=0.0016). There was also significant difference between mild and moderate HIE groups in terms of plasma level of HO-1 (91.23 ± 3.35 pg/ml Vs. 105.5 ± 5.76 pg/ml, $P < 0.035$), while there was no significant difference between sever and moderate neonatal HIE groups (P=0.14)

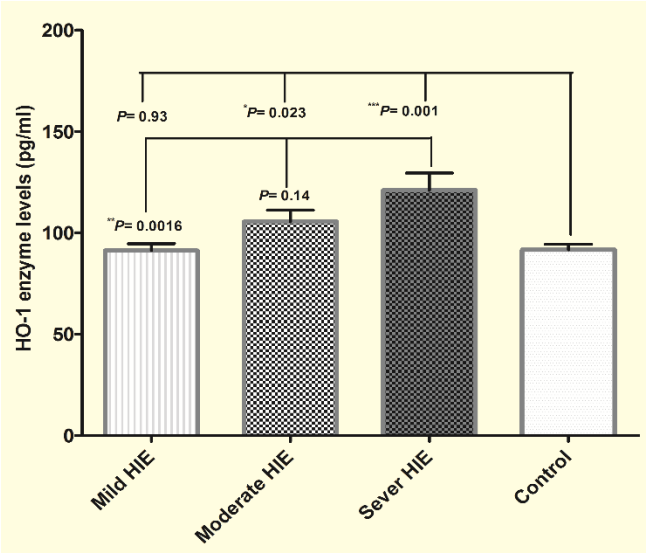

Figure 2. Comparison of plasma levels of HO-1 enzyme in neonatal HIE subgroups according to Sarnat staging.

HIE: Hypoxic Ischemic Encephalopathy, HO-1: Heme-Oxygenase-1, Data represent mean ± SEM. The values of P<0.05 were considered significant.

Discussion

Among three isoforms of heme oxygenase (HO-1, 2, and 3), HO-1 is the only isoform with limited expression under typical situations and is induced by various physiological stimuli (19). Several reports have shown that multiple cellular stresses and triggers, including oxidative stress, inflammatory cytokines, ischemia, and hypoxia, upregulate the expression of HO-1 in different types of cells, suggesting an essential role for

this enzyme in tissue protection (11, 20-22). In an animal model study of Alzheimer's disease, Gupta *et al.* claimed that HO-1 can be considered a therapeutic target for brain damage (23). In another animal study using mice, Ding *et al*. showed that neuroprotective effects of several agents in ischemic brain injury involve the HO-1 defense pathway (24). Although extensive animal studies have revealed the importance of HO-1 in tissue protection, especially in the brain, there is still very little scientific understanding of the crucial role of HO-1 in the pathophysiology of human diseases such as neonatal HIE.

The present study was designed to evaluate the plasma level of HO-1 in healthy and HIE-affected newborns. We also assessed the relationship between HO-1 enzyme level and clinical severity of neonatal HIE and found significantly higher plasma level of HO-1 in neonatal HIE group compared *with* healthy full-term newborns (Figure.1). This result is consistent with Bergeron's findings (14) showing that perinatal hypoxic-ischemic induces HO-1 in newborns' rat brains. Our results also indicated differences in plasma levels of HO-1 based on various stages of neonatal HIE; that is, its level in severe and moderate neonatal HIE patients was significantly higher than that in mild neonatal HIE patients (P<0.05) (Figure.2). This finding agrees with Dani *et al*. (13) study suggesting that the concentration of HO-1 reflects the severity of HIE in neonates. Zhao *et al*. (25) in an animal model of HIE, showed that upregulation of HO-1 expression following argon-hypothermia treatment significantly reduces cell death in oxygen-glucose deprivation-exposed cortical neurons.

It is now well established from various studies that the pathological damage in neonatal HIE resulting from hypoxemia/ischemia is due to the deprivation of oxygen and glucose supply, which causes a primary energy failure and initiates a cascade of biochemical events (6, 26). On the other hand, it has previously been observed that deprivation of oxygen and glucose is a potent inducer of HO-1 expression in many cell types (27). Therefore, increased plasma level of HO-1 during neonatal HIE may play a cytoprotective role in the resolution of neuropathology in the brain. In support of this possibility, Nitti *et al*. (28) have shown that up-regulation of HO-1 represents a powerful mechanism of cell adaptation to stress. Its antioxidant and anti-inflammatory properties are mainly due to the biological activity of metabolic products.

Conclusion

According to the results of this study, our preliminary findings suggested that HO-1enzyme may be associated with the pathophysiology and clinical severity of neonatal HIE. These findings may be somewhat limited by single center study design, small sample size, and the method used for the measurement of HO-1. Thus, as perspective, the further research should be undertaken to investigate the correlation of plasma level of HO-1 enzyme at birth with the multi-organ dysfunction and abnormal neurodevelopmental outcomes in full-term newborns with HIE as a multicenter study with sufficient sample size.

Conflict of interests

The authors declare that they have no conflict of interests.

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