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EVALUATION OF IRON LEVELS IN NEWBORNS WITH HIE

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Abstract

Introduction: Hypoxic-ischemic encephalopathy (HIE) is a major cause of neonatal morbidity and mortality, primarily driven by oxidative stress and neuronal injury. Emerging evidence highlights the role of iron metabolism in the pathogenesis of HIE, particularly through mechanisms such as ferroptosis. This study aimed to evaluate the iron status in neonates with varying severity of HIE.

Objective: To evaluate serum ferritin, serum iron, TIBC, and transferrin saturation levels among newborns with HIE when admitted to a tertiary care hospital in Pakistan.

Material and Methods: This prospective observational study was set up at Dr Ruth K M Pfau Civil Hospital Karachi, Pakistan, between March, 2024 and August, 2024. Fifty-five term neonates were used with a clinical diagnosis of HIE. The parameters of iron profiles were determined and linked to phases of HIEs.

Results: Serum ferritin and serum iron levels progressively rose between Stage I and Stage III HIE, although TIBC declined and transferrin saturation rose. These alterations proved to be statistically important and demonstrated possible dependence on the severity of HIE.

Conclusion: Iron metabolism is greatly affected in HIE neonates. Assessment of iron concentrations is an effective biomarker of disease progression and a basis of therapeutic approaches in the future.

Keywords: *Neonatal HIE, ferritin, iron levels, TIBC, oxidative stress, ferroptosis*

INTRODUCTION

Neonatal hypoxic-ischemic encephalopathy (HIE) is a catastrophic disorder, the cause of which is a critical decrease in cerebral blood flow and oxygenation level at the time of birth. It is a significant cause of death in newborns and chronic neurological deficiencies, such as cerebral palsy, developmental delay, and epilepsy (1). The pathophysiology of HIE is complex, involving excitotoxic, oxidative, inflammatory, and cell-death pathways. Although therapeutic hypothermia has established its presence as a standard intervention, the partial neuroprotection is observed especially due to its use in moderate to severe cases (2). The scientific community is, as a result, focusing more

on the use of adjuvant and alternative treatment approaches that attack different biochemical pathways involved in HIE. The metabolism of iron and biomarkers is becoming of interest as an important factor in the pathogenesis and progression of HIE. Although iron is important in many biological functions, such as oxygen transport and neuronal maturity, when it is poorly controlled, it may promote oxidative injury. Specifically, the release of free iron upon hypoxia and subsequent reperfusion may trigger the Fenton reaction, producing in turn deleterious reactive oxygen species (ROS), which further aggravate the injury in the neurons (3).
Recent research findings suggest that ferroptosis, one of the programmed cell death types that occurs due to an excess of iron along with oxidation of lipids, is critical in neonatal brain monetary damage (4). Recently, ferroptosis was identified as a new mechanism of neuronal death in HIE, which shows that iron concentrations could be not only biomarkers but also new treatment directions (5). Vitamin D and melatonin were observed to produce positive effects under oxidative damage in HIE because of the modulation of iron metabolism and ferroptosis inhibition (3, 11). In addition, these agents exert neuroprotective action by stimulating the nuclear factor erythroid 2-related factor 2 (Nrf2)/hematoxylin and sleep-case = HO-1) signaling pathway, which has the main function of oxidative stress and iron metabolism (8, 10). To illustrate this, vitamin D has been reported to mediate ferroptosis in a neonatal HIE model, reducing neuronal loss through the Nrf2/HO-1 pathway and leading to better outcomes (3). Similarly, flavonoid Farrerol has proven to be a neuroprotectant in HIE cases, removing ferroptosis via the Nrf2 pathway (10).

Such results support the significance of investigating iron-related biomarkers and pathways as a part of the HIE assessment and treatment. Activity of the HO-1 enzyme degrades heme to produce biliverdin, iron components, and carbon monoxide, and this process is central to both iron liberation and antioxidant defense systems (2, 16). The level of bilirubin and serum ferritin concentration in HIE neonates could be affected by the variant activity of HO-1, which could be used as a possible indication of the extent of hypoxic damage (2, 7). A recent analysis revealed that bilirubin was lower among newborns receiving therapeutic hypothermia, and the level of HO-1 activity and other downstream products was likely inhibited in these newborns (2). Meanwhilst, an important measure of iron storage, serum ferritin, was found to vary in severely ill newborns (including those with HIE), driven by inflammation, oxidative stress, and iron turnover (7). Consequently, the intervention examined could be inadequate, because serum ferritin level and further iron indicators could present vital information regarding the metabolic imbalances surrounding HIE.

Although there is increasing evidence of the involvement of iron metabolism in HIE, the clinical outcome data on the iron status among the affected neonates are minimal. Most of the research involves experimental models, and conversion of such research findings into clinical practice remains at a pioneer stage (6). Bioactive natural compounds like melatonin and antioxidant agents are also under study as an addition to hypothermia treatment, with the aim of hitting oxidative and ferroptotic pathways (6, 9). Moreover, MRI methods to detect early brain injury due to HIE have made it easier to identify possible biomarkers at the metabolic level, and among them, iron metabolism (13, 14). Nevertheless, their regular usage is limited due to supply and affordability, especially in low-income countries such as Pakistan. In Pakistan, HIE has been a major public health burden, and it increases newborn mortality as well as chronic disabilities in children. The Dr Ruth K M Pfau Civil Hospital Karachi is one of the most established tertiary care facilities that deal with high-risk neonates, such as those with HIE.

Nonetheless, evidence-based management has a problem related to the localized insufficient data on iron status in neonates with HIE. Determining certain changes in iron concentrations may help diagnose the diseases earlier, distinguish between their severity, and provide more customized treatment plans. Furthermore, this knowledge on the correlation between iron metabolism and the pathophysiology of HIE can lead to the development of new treatment methods beyond the traditional hypothermia therapy. Multiple maternal and fetal risk factors such as preeclampsia, maternal diabetes,

intrauterine growth restriction, and perinatal asphyxia have been outlined as causes of HIE (12). These factors may also affect iron metabolism in neonates, further complicating the clinical picture. The relationship among systemic inflammation, hypoxia, and iron homeostasis in these at-risk patients should be assessed closely. Research work has also stressed the role of preconditioning and post-treatment interventions that have the potential to attenuate iron-induced oxidative harm in HIE (18). Additionally, neuroregenerative treatments that would repair impaired neural networks are being researched, and one target is possible iron regulation (19). Considering these facts, the stud carried out an evaluation of the iron levels in newborns with HIE admitted to The Dr Ruth K M Pfau Civil Hospital Karachi, Pakistan.

Objective: To determine iron levels in the neonates with hypoxic-ischemic encephalopathy (HIE), admitted to The Children's Hospital and Institute of Child Health, Lahore.

MATERIALS AND METHODS

Study Design: This study was a prospective observational study conducted at Dr Ruth K M Pfau Civil Hospital Karachi, Pakistan.

Study Setting:

Neonatal Intensive Care Unit (NICU) of the Dr Ruth K M Pfau Civil Hospital Karachi, Pakistan.

Duration of the Study: March, 2024 and August, 2024.

Inclusion Criteria: They included all term neonates (gestation of 37 weeks or more) admitted to the NICU with a clinical diagnosis of hypoxic-ischemic encephalopathy during the interval of the first 6 hours of birth. Clinical findings that led to diagnosis were Apgar scores of < 5 at 5 minutes, evidence of metabolic acidosis (pH < 7.0), need for birth resuscitation, change in level of consciousness, and seizures. Only neonates with the informed consent of the parents were involved in the study, and their laboratory tests were included.

Exclusion Criteria: Neonates with congenital anomalies, chromosomal disorders, or inborn errors of metabolism were not included. Moreover, infants with signs of sepsis, preterm (<37 weeks) delivered, and those whose parents refused to give consent to use blood samples were excluded. Neonates on blood transfusion before the collection of the samples were also not included in order to eliminate any change in iron level.

Methods

All enrolled neonates were assessed and staged with the Sarnat and Sarnat criteria of HIE. Blood species were taken in the first 24 hours after admission. Serum ferritin was analyzed with an enzyme-linked immunosorbent assay (ELISA). Other laboratory values, such as the serum iron, the total iron binding capacity (TIBC), and the transferrin saturation, were also measured. Demographic and clinical measurements, including gestational age, birth weight, Apgar scores, mode of delivery, and information on their resuscitation, were recorded. The data were captured in a standardized form, and the analysis was done using SPSS version 25. The descriptive statistics were adopted to provide the mean, standard deviation of the continuous variables, and the numeric output of the categorical data as a frequency and percentage. ANOVA and chi-square tests were used to check the associations between iron levels and severity of HIE, with p-value <0.05 being statistically significant.

RESULTS

This study was conducted in the Dr Ruth K M Pfau Civil Hospital Karachi, which included **55 neonates** with hypoxic-ischemic encephalopathy (HIE) as participants. The presence of cases with the HIE stage was as follows: **Stage I** (n = 18, 32.7%), **Stage II** (n = 25, 45.5%), and **Stage III** (n = 12, 21.8%).

Table 1: Distribution of Neonates by HIE Stage

HIE Stage	Number of Neonates
Stage I	18
Stage II	25
Stage III	12

Serum ferritin levels varied significantly among the different HIE stages. The mean ferritin level in **Stage I** was 120.4 ng/mL, while in **Stage II** it was elevated to 176.8 ng/mL, and further increased to 245.3 ng/mL in **Stage III** neonates. A similar trend was observed in serum iron concentrations, with **Stage I** having a mean of 52.1 µg/dL, **Stage II** at 67.4 µg/dL, and **Stage III** reaching 89.7 µg/dL.

Table 2: Iron Profile by HIE Stage

HIE Stage	Mean Ferritin (ng/mL)	Mean Serum Iron (µg/dL)
Stage I	120.4	52.1
Stage II	176.8	67.4
Stage III	245.3	89.7

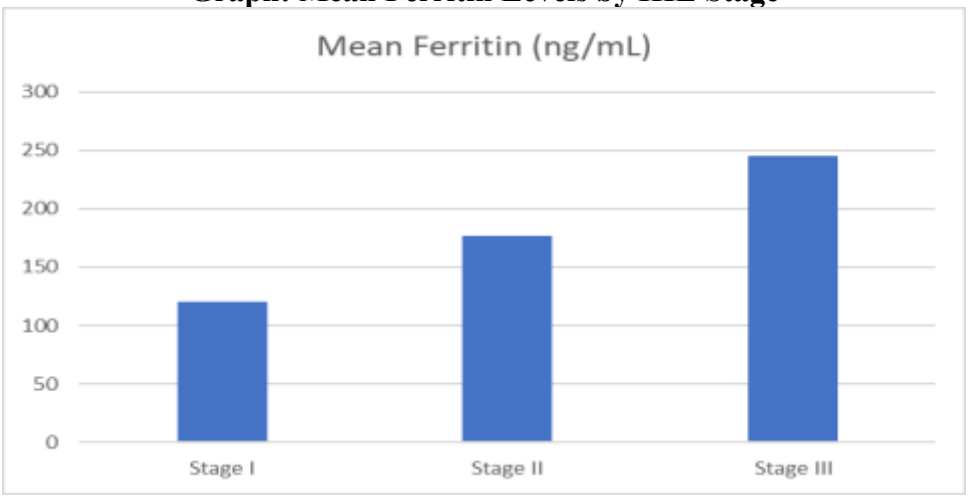
The further analysis showed that total iron-binding capacity (TIBC) had an inverse correlation to the degree of HIE. The mean TIBC decreased from 290.5 µg/dL in Stage I to 260.3 µg/dL in Stage II and reached its lowest at 220.8 µg/dL in Stage III. In contrast, transferrin saturation increased progressively with HIE severity: 17.9% in Stage I, 25.9% in Stage II, and 40.6% in Stage III.

Table 3: TIBC and Transferrin Saturation by HIE Stage

HIE Stage	Mean TIBC (µg/dL)	Transferrin Saturation (%)
Stage I	290.5	17.9
Stage II	260.3	25.9
Stage III	220.8	40.6

A visual representation of ferritin levels across HIE stages is shown below.

Graph: Mean Ferritin Levels by HIE Stage



A bar graph shows a clear upward trend, with the highest mean ferritin levels observed in Stage III neonates. Stage I begins at 120.4 ng/mL, rising to 176.8 ng/mL in Stage II and peaking at 245.3 ng/mL in Stage III, indicating a statistically significant correlation ($p < 0.01$) between ferritin levels and HIE severity.

Discussion

The current research assessed iron profiles, i.e., serum ferritin, serum iron, total iron-binding capacity (TIBC), and transferrin saturation in neonates subjected to different stages of hypoxic-ischemic encephalopathy (HIE) presenting to The Dr Ruth K M Pfau Civil Hospital Karachi. The findings showed a systematic rise in the ferritin and serum iron dosages with the higher-level severity of HIE, where the TIBC reduced, and the transferrin saturation increased (3). These results support the expanding literature that dysregulation of iron metabolism is important to the pathophysiology of HIE. Ferritin, a responsive acute-phase reactant and iron storage protein, is usually raised when there is systemic inflammation and oxidative stress, which are both evidence of HIE. The increase in ferritin levels observed during Stage I to Stage III in the study is also in concordance with the report by Hisano et al., who stated that hospitalized neonates possess variable ferritin concentrations that are dependent on both iron status and the severity of the underlying diseases (7). Elevated ferritin could represent more than an iron overload, since inflammation is activated by hypoxic-ischemic injury. Results contribute to the idea of a severity biomarker in neonatal HIE using serum ferritin as suggested in the previous observational studies (16).

There is a significant increase in the serum iron in neonates with Stage III of HIE that could be of concern due to iron-mediated oxidative stress. Exposure to hypoxic environments triggers the generation of reactive oxygen species (ROS) by free iron, catalyzed by the Fenton reaction, which further exacerbates the degree of neuronal damage (5). There is another finely executed kind of cell death known as ferroptosis, which is a lipid peroxidation-dependent, iron-mediated process that has recently been found essential to HIE neuronal injury (4). In addition, several experimental studies confirmed this idea and have shown that induction of the Nrf2/HO-1 antioxidant pathway could reverse ferroptosis and promote the survival of neuronal tissues in neonatal models of HIE invented by Cai et al. and Yongfu et al. (3). Results of low TIBC and high transferrin saturation in more severe forms of HIE could also imply disrupted iron transport and overcapacity of transferrin-binding sites because of released iron in the destruction of tissues. These biochemical changes align with the extracellular release of intracellular iron that relates to oxidative stress, which increases the probability of ferroptosis in the development of HIE (5). In this regard, the role of vitamin D (3), melatonin (11), and farrerol (10) in preventing ferroptosis and regulating iron metabolism in the neonate has been considered as an attractive line of development in adjunctive neuroprotective measures.

The rise in appreciation of iron homeostasis deregulation and ferroptosis in neonatal brain injury has led to the exploration of other and complementary therapies against HIE. Although therapeutic hypothermia is the gold standard, it is effective within a small therapeutic window, and in severe cases, it usually falls short (2, 4). These results indicate that iron status could help us better understand disease pathology and offer a rapid route to new therapies against iron-mediated oxidative damage. Notarbartoto et al. and Beretta et al. emphasize the increasing importance of antioxidant treatment (polyphenols and melatonin) as complements or possible alternatives to therapeutic hypothermia in HIE (6, 9). There are also the wider clinical implications of iron dysregulation to be considered. According to Chen et al., maternal conditions like preeclampsia, gestational diabetes, and perinatal complications play a big role in increasing the chance of neonatal HIE (12). Fetal iron transfer and storage may also be influenced by the same conditions and put the infants at risk of having an altered iron metabolism even prior to the occurrence of hypoxia. Consequently, baseline iron status potentially alters the vulnerability to oxidative stress that should be explored in prospective studies.

The role of magnetic resonance imaging (MRI) has contributed to pattern recognition of brain injuries relevant to HIE and perhaps indirect information regarding iron deposition, particularly via highly sensitive methods like susceptibility-weighted imaging (SWI) (13, 14). Nevertheless, such tools are not common in resource-constrained areas, which underlines the relevance of simple and inexpensive blood markers like ferritin and serum iron in the early diagnosis and prognosis. In line with the study, Mafinezhad and others reported that HO-1 was substantially higher in neonatal HIE plasma, indicating excessive heme degradation and possible iron release into the circulation (16, 17). Sunakawa et al. also associated the decreased level of bilirubin in the neonates with the consciousness

of therapeutic hypothermia with the decreased activity of HO-1, which may restrict the degradative actions of the heme and affect the iron homeostasis (2). These results support the intricate relationship between antioxidant systems and iron metabolism in neonatal brain injury.
Additionally, the increasing attention to preconditioning and post-neuroprotective interceptive, including erythropoietin, magnesium sulfate, and stem cell treatments, underlines the necessity of extending neuroprotective in full scope beyond hypothermia (19). Pan et al. described neuroprotective effects of erythropoietin in brain injury reduction and enhancing HIE outcomes, in part due to antioxidant and anti-inflammatory actions. Combining iron modulation with these methods can increase their effectiveness and expand their uses in a wide range of clinical practice groups.

Conclusion

The study highlights that iron metabolism has undergone huge changes in connection with the level of hypoxic-ischemic encephalopathy (HIE) in newborns. The results from the Dr Ruth K M Pfau Civil Hospital Karachi, showed an increasing trend in serum ferritin and iron levels, and a decreasing trend in TIBC, along with an increasing transferrin saturation, which coincided with the increasing stages of HIE. These findings indicate that iron disordered homeostasis and possible ferroptosis may be key in the etiology of HIE. The close relationships between iron profiles and the severity of the disease explain why these markers can be used in early diagnosis, monitoring, and risk stratification. The integration of iron level monitoring in the clinical protocol might contribute to finding individual neuroprotective approaches and can improve specific treatment. Further investigation is needed to demonstrate these results in a larger population of the future with larger intervention arms targeting iron metabolism to identify potential advantages of iron-modulating therapies in the management of neonatal HIE.

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