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ORIGINAL ARTICLE

Assessing Ferrous Level in Neonates Suffering from Hypoxic Ischemic Encephalopathy

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ABSTRACT

Aim: To assess the ferrous level in Hypoxic Ischemic Encephalopathy.
Methodology: Eighty four neonates from tertiary care hospital had been included. Neonates were classified as hypoxic on the basis of Sarnat and Sarnat scoring. 34 healthy neonates served as control. Among 50 patients two groups were made each containing 25 neonates. Group 1 comprising of 25 neonates of hypoxic ischemic encephalopathy of mild degree. Group 2 comprising of 25 neonates suffering from moderate to severe encephalopathy.
Results: Serum ferrous was significantly elevated in neonates suffering from Hypoxic Ischemic Encephalopathy.
Conclusion: The significant elevation of serum ferrous in hypoxic neonates reiterates the key role of serum ferrous in neonates with hypoxic ischemic encephalopathy.
Keywords: Ferrous, oxidative stress.

INTRODUCTION

The requirement of brain for oxygen is high concentration as it has immature cells, free radicals and low concentration of antioxidant enzymes. Perinatal asphyxia is a leading cause of neonatal mortality and disability. They are visual impairment, learning impairment, epilepsy, mental retardation and cerebral palsy.[1]It is one of the causes in accumulation of iron in the brain of neonates of the mammals. This result in neurobehavioural disturbances which are impaired learning and abnormal alertness during life.[2] after hypoxia, oxidative stress generates the release of oxygen and nitrogen derived free radicals leading to excitotoxicity and acidotoxicity. There is calcium over loading, ionic imbalance, inflammation and necrosis. Superoxide and hydrogen peroxide reacts with iron via Haber-Weiss reaction to produce hydroxyl radicals[3]. A delicate imbalance between pro-oxidant and anti-oxidant tips towards oxidative stress[4].

World Health Organization (WHO) states that approximately 4 million babies die each year in neonatal period. Ninety eight percent of these deaths occur in the developing countries. Twenty nine percent of these are due to perinatal asphyxia and birth injuries[5]. But neonatal hypoxic ischemic encephalopathy has become an infrequent condition in developed countries.[6]Studies assessing asphyxiated insult in neonates are not available in developing countries but it is probable that intrapartum is the major cause[7].

The most important drawback for post-asphxial hypothermia is the small therapeutic window. Its timing is within 6 hours of birth in which treatment should be initiated.[8]Current treatments are restricted to management of complications and supportive care to the patients but there is limited data available regarding cellular and molecular events leading to hypoxic ischemic encephalopathy[9].

Iron is a metal required for basic biochemical functions in the body. Elemental iron becomes part of the porphyrin ring of hemoglobin, myoglobin and cytochromes. It is required for the functions of neurotransmitter[10]. It is also essential for myelin formation, development of dendritic connections. It is involved in functioning of enzyme systems for the regulation of cellular energy. [11]Neonatal nervous system has high concentration of free iron[12].

Ferrous is released in presence of superoxide, acidic pH, ascorbate and cataecholamines during hypoxia[13]. It leads to the formation of free radicals through Fenton reaction[14]. NPBI is a pro-oxidant which can convert hydrogen peroxide into hydroxyl radicals. These free radicals can cause injury to the brain[15]. Ferrous ions also catalyze the alkoxyl radicals production in the presence of hydroperoxide (ROOH), which leads to chain reaction[16]. It enhances the formation of others ROS and lipid peroxidation[17,18]. These ROS can further initiate lipid and protein oxidation and Deoxyribonucleic acid (DNA) modification[19].

The objective of the study was to assess the ferrous level in Hypoxic Ischemic Encephalopathy.

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METHODOLOGY

This study was approved by of UHS Lahore. It was conducted in collaboration with Services Hospital Lahore. This Descriptive analytic study was carried out on eighty four neonates admitted in neonatal unit of tertiary care hospital had been included. Out of these 84 neonates, the 34 healthy neonates served as control. The 50 neonates were divided into two groups each containing 25 neonates. Group 1 comprised of 25 neonates suffering from hypoxic ischemic encephalopathy of mild degree where as Group 2 comprised and base deficit >12mmol/l) Multiorgan involvement. Abnormal neurology or clinical seizures. Demographic data was collected on a proforma along with history of neonate. They were diagnosed on the basis of APGAR sore of less than 3 at 1minute and at 5min less than 7. Metabolic acidosis is evident in umbilical arterial blood, or neonatal blood gas samples (pH <7 Any condition that alters the level of glutamic acid and ferrous e-g, Intra uterine growth retardation (IUGR), gross structural abnormalities, Septic shock and birth trauma were excluded.

Neonatal blood samples were obtained from arteries of neonate. Arterial blood gas analysis of blood was done from laboratory of Services hospital Lahore. Serum ferrous was measured by colorimetric method for iron determination without deproteinization using a commercially available kit (Wiener Lab).

RESULTS

The collected data was entered into SPSS version 16. The quantitative measures include ferrous and presented as mean and standard deviation. The levels of iron were compared within three groups of neonates by applying ANOVA for significance. A *p* value of less than 0.05 was taken as significant. Comparison between groups and within the groups was done by applying Post Hoc Test.

ANOVA

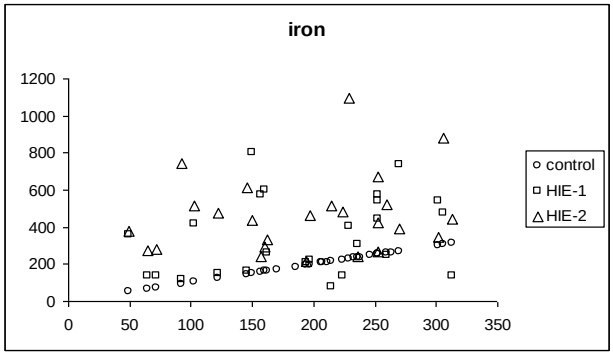
Table 1: Serum Iron comparison with normal

Groups	Sum of squares	Mean square
Between groups	1026452.8	513226.401
Within groups	2305139.5	28458.512
Total	3331592.3	

Significance 0.000

The correlation between serum ferrous levels in hypoxic and control was found to be significant (p= 0.000)

Figure: Serum Iron in healthy neonates versus neonates suffering from Hypoxic Ischemic Encephalopathy



Post Hoc Tests for Iron: When we compare the results after applying post Hoc, a significance of 0.003 was found between normal neonates and those with HIE-1, and a significance of 0.000 when normal were compared with those of grade2 &3. But when HIE1 and HIE2&3 were compared with each other than a significance of 0.05 was found which is not significant. Table shows groups with their mean difference, Std. Error and significance.

Table 2: Comparison of serum Iron within the groups

(I) Groups	(J) Groups	Mean Difference (I-J)	Std .Error	Significance
Normal neonates	HIE-1	-152.50914*	44.44497	0.003
	HIE 2 &3	-263.04794*	44.44497	0.000
HIE-1	Normal	152.50914*	44.44497	0.003
	HIE 2& 3	-110.53880	47.71458	0.059
HIE2&3	Normal	263.04794*	44.44497	0.000
	HIE-1	110.53880	47.71458	0.059

DISCUSSION

Ferrous has an important role to play in hypoxic ischemic encephalopathy and we have found significant higher levels of free iron. Mean value of Iron in control was1.9826E2±11.90244 while that of the HIE grade1 was3.5077E2±42.29501*** and grade 2-3 was 461.310±42.2861*** (Fig. 1). Our values of Ferrous were higher even in 3 to 4 control .These may be related to some of the preterm neonates in study as preterms have higher non protein bound iron levels. Excess of free iron showing deficient iron metabolizing and binding capacity favors oxidant stress in premature infants[17].

Scattered graph of results shows neonates suffering from severe hypoxia have higher levels of serum ferrous than those suffering from mild to moderate asphyxia. Graph indicates a positive correlation between degree of hypoxia and concentration of ferrous. X-axis represents number of patients where as y axis concentration of ferrous. After applying ANOVA a significant p value of 0.000 has been found.

In relation to the oxidative stress Shouman et al have demonstrated that concentration of non protein bound iron (NPBI) in serum and CSF were elevated but total iron binding capacity was decreased in neonates suffering from hypoxic ischemic encephalopathy[18]. This study has shown almost similar results like ours with a p value of .001.Shahid reported that reperfusion after hypoxic-ischemic (HI) induced increased production of free iron which had produced highly reactive hydroxyl radical[19].

According to latest estimates by World Health Organization (WHO), approximately 4 million babies die each year before they reach the age of one month. Ninety eight percent of these neonatal deaths take place in the developing countries. Perinatal asphyxia and birth injuries together contribute to almost 29% of these deaths[6]. But the neonatal hypoxic ischemic encephalopathy has become an infrequent condition in developed countries[7]. Studies assessing the timing of asphyxiated insult in neonates are not available in developing countries but it is likely that intrapartum causes account for a larger proportion of cases[18].

The most important drawback for post-asphxial hypothermia is the small therapeutic window. The timing for this window is within 6 hours of birth in which treatment should be initiated. Current treatments are restricted to management of complications and supportive care to the patients but there is limited data available regarding cellular and molecular events leading to hypoxic ischemic encephalopathy.

CONCLUSIONS

Our study leads us to suggest that increased levels of ferrous are associated with oxidative stress in neonates suffering from hypoxic ischemic encephalopathy. It provides a data suggesting a relationship between rising levels of serum ferrous and severity of hypoxic ischemic encephalopathy. It provides information that serum ferrous leads to production of free radicals. Clearly on larger scale further studies should be designed to establish a correlation between ferrous and oxidative stress.

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REFERENCE

1. Banupriya C, Ratnakar, Doureradjou P, Mondal N, Vishnu B. Can urinary excretion rate of malondialdehyde, uric acid and protein predict the severity and impending death in Perinatal asphyxia? Clin Biochem.2008; 41:968-73.Epub 2008
2. Roqalska J, Caputa M, Wentowska K, Nowakowska A. Stress-induced behaviour in adult and old rats:effect of neonatal asphyxia,body temperature and chelation of iron. J Physiol Pharmacol.2006; 57:17-34.
3. Kumar A, Ramakrishna SV, Basu S, Rao GR. Oxidative stress in Perinatal asphyxia. Pediatr Neurol.2008; 38:181-5.
4. Solovieva EY, Chipova DT. From the conception of oxidaizing stress to the conception of cell signaling modulation.Im S S.2015;115:105-111.
5. Agarwal R, Jain A, DeorariAK, Paul VK. Post- resuscitation management of asphyxiated neonates. Indian J Pediatr 2008; 75:175-80.
6. Garcia-Alix A, Martinez-Biarqe M, Diez J, Gaya F, Quero J. Neonatal hypoxic ischemic encephalopathy:incidence and prevalence in the first decade of 21st century. An Pediatr (Barc) 2009; 71:319-26
7. Azra Haider B, Bhutta ZA. Birth asphyxia in developing countries: Current status and public health implications. Curr Probl Pediatr Adolesc Health Care.2006; 36: 178-88.
8. Torrance HL, Benders MJ, Derks JB, Rademaker CM, Bos AF, Van Den Berq P, Lonqini, Buonocore G, Veneqas M, Baquero H, Visser H, Van Bel F. Maternal allopurinol during fetal hypoxia lowers cord blood level of the brain injury marker S-100B. Pediatrics.2009; 124:350-7.
9. Sullivan SM, Bjorkman ST, Miller SM, Colditz PB, Pow D. Structural remodeling of matter astrocytes in the neonatal pig brain after hypoxic/ischemia.Glia.2009Jul 15
10. Michael K, Georgieff. Iron in the brain. Its role in development and injury. Neoreviews.2006.
11. Selim MH, Ratan RR. The role of iron neurotoxicity in ischemic stroke. Aqeing Res Rev.2004; 3:345-53.
12. Ferriero DM . Oxidant mechanisms in neonatal hypoxic ischemia. Dev Neurosci.2001;23:198-202.
13. Bishop GM, Robinson SR. Quantitative analysis of cell death and ferritin expression in response to cortical iron: implications for hypoxic-ischemia and stroke. Brain Res.2001; 907:175-87.
14. Andresen JH, Saugstad OD. Effect of nicotine infusion on striatal glutamate and cortical non-protein-bound iron in hypoxic new born piglets. Neonatology. 2008; 94:284-92.
15. Yu T, Kui LQ, Ming QZ. Effect of asphyxia on non-protein bound iron and lipid peroxidation in new born infants.Dev Med Child Neurol.2003; 45:24-7.
16. Oqihara T, Hirano K, Oqihara H, Misaki K, Hiroi M, Morinobu T, Kim HS, Oqawa S, Ban R, Haseqawa M, Tamai H. Non protein bound transition metals and hydroxyl radical generation in cerebrospinal fluid of new born infants with hypoxic ischemic encephalopathy. Pediatrl Res. 2003;53:549-9.
17. Ansari MA, Roberts KN, Scheff SW.A time course of NADPH oxidase up regulation and endothelial nitric oxide synthase activation in the hippocampus following neurotrauma. Free Rdical Biology and Medicine.2014; 77:21-29.
18. Angeloni C, Parta C, Dalla Sega FV, Piperno R, Hrelia S. Traumatic brain injury and NADPH oxidase: A deep relationship. Oxid. Med. Cell. Longev.2015; 2015.
19. Hamrick S E G, Mcquillen P S, Jiang X, Mu D. A role for hypoxia _inducible factor-1 in deforaxamine neuroprotection.neulet 2004; 379:96-100.
20. Buonocore G, Perrone S, Bracci R. Free radicals and brain damage in new born. Biol Neonate.2001;79:180-6.
21. Shouman BO, Mesbah A, Aly H. Iron metabolism and lipid peroxidation products in infants with hypoxic ischemic encephalopathy .J Perinatol.2008;28:487-91.
22. Shahid M, Van Bel F, Steendijk P, Dorrepaal CA, Moison R, Van Der Velde ET, Baan J. Effect of deferoxamine on post-hypoxic –ischemia reperfusion injury of the new born lamb heart.Biol Neonate.1999;75: 239-49.