ORIGINAL ARTICLE

Association of the Level of Glutamic Acid and Iron with Severity of Hypoxic Ischemic Encephalopathy in neonates

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ABSTRACT

Background: Dailymillions of neonates suffer from birth asphyxia worldwide, due to poor quality of mother and child health care. A large number of living neonates are terminated due to hypoxia however those who survive suffer from developmental delay, mental retardation, epilepsy and cerebral palsy. Glutamate and ferrous damages the brain by generating free radicals, which causes damage to lipids, proteins and nucleic acid of these fragile beings that are just beginning to breathe in the world.

Methods: Eighty four neonates admitted in neonatal unit of 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 suffering from hypoxic ischemic encephalopathy of mild degree. Group 2 comprising of 25 neonates suffering from moderate to severe encephalopathy.

Results: Serum levels of glutamate and Iron were significantly raised in this study indicating the oxidative stress by glutamate and iron in pathogenesis of hypoxic ischemic encephalopathy

Conclusion: Glutamate and Iron are powerful neurotoxic agents so potential therapeutic strategies should be designed for the neonates suffering from hypoxic ischemic encephalopathy in minimizing the levels of glutamic acid by use of antagonist, chelation therapy in minimizing the level of free total iron. The use of antioxidant therapy is also recommended.

Key words: Hypoxic ischemic encephalopathy, glutamic acid, iron, oxidative stress

INTRODUCTION

Encephalopathy is a clinically defined syndrome of disturbed neurological functions in which entire brain does not receive enough oxygen, during the first few days of life. This results into an imbalance in metabolic demand and cellular energy supply leading to difficulty with initiating and maintaining respiration depression of tone and reflexes, altered level of consciousness and seizures. Neurological sequelae are known to delay motor development, mental retardation, learning disabilities, seizures disorders and cerebral palsy.[1]Between 2-4/1000 full term newborns suffer from asphyxia at or shortly before birth. Approximately 15% to 20% of such asphyxiated neonates die and among the survivors 25% exhibit permanent damage or disability[2]. Glutamate excitotoxicity is the major mechanism in causing neonatal death after hypoxic ischemic insult[3]. Glutamate mediated free radical generation after Ca^{+2} accumulations is one of the major mechanisms causing cell death in hypoxic-ischemic brain injury[4]. Oxidative stress occurs when production of reactive oxygen species (ROS) exceeds beyond the body's ability to detoxify these ROS. ROS include superoxide radicals, hydrogen peroxide and hydroxyl radicals. They are transient in nature. ROS stimulate opening of L-type voltage sensitive calcium channels (L-VSCC) resulting in an increased intracellular calcium further worsening the situation[5]. They attack polyunsaturated fatty acids of plasma membrane resulting in increase membrane permeability[6].

After the hypoxic insult, accumulation of the ferrous in immature brain occurs within 4 hours where as in mature ischemic changes are not seen till 4-8 weeks of insult[7]. Release of Fe^{2+} from ferritin can be accomplished by superoxide, acidic pH, ascorbate and cataecholamines which are abundant in extracellular fluid of brain during hypoxia[8]. It contributes to the formation of free radicals through Fenton reaction[9]. These ROS can further initiate injury through lipid and protein oxidation and Deoxyribonucleic acid (DNA) base modification[10].

METHODOLOGY

This study was approved by Advanced Study and Research Board of University of Health Sciences Lahore.It was conducted at the University of Health Sciences (UHS) Lahore in collaboration with Services Hospital Lahore.This Descriptive analytic study was carried out on eighty four neonates admitted in

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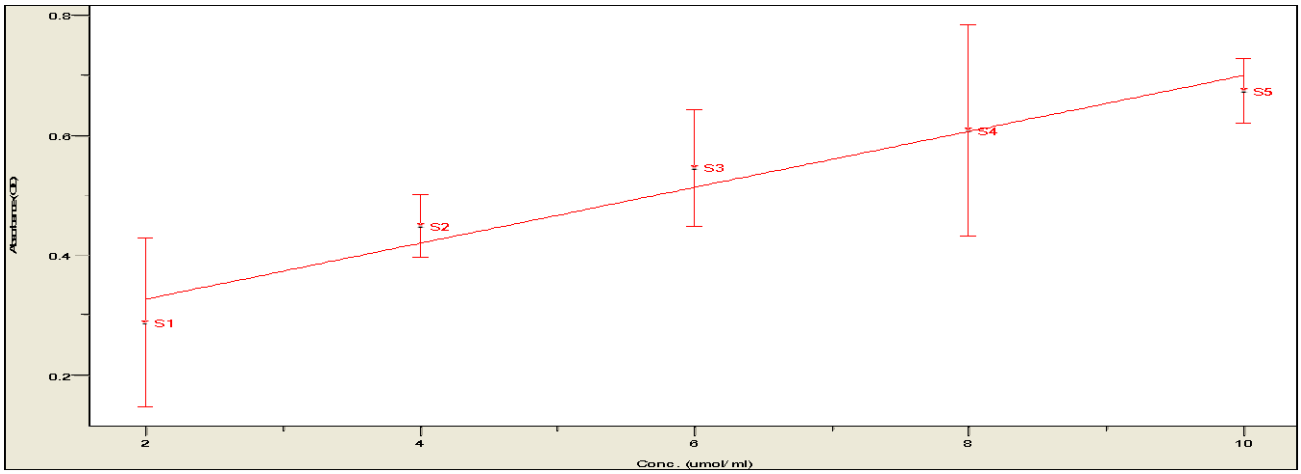
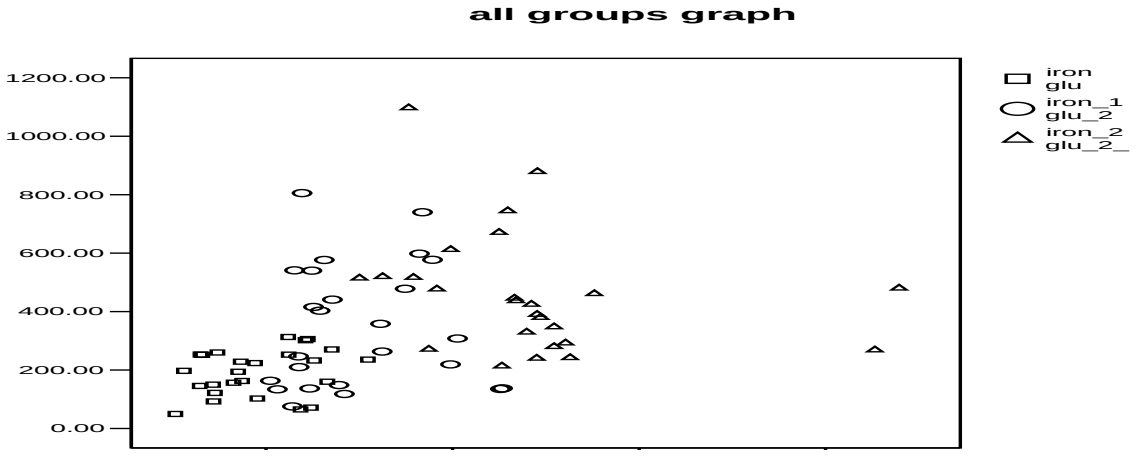
neonatal unit of tertiary care hospital had been included. Out of these 84 neonates, the 34 healthy neonates served as control. The 50 neonates were divided into two groups each containing 25 neonates. Group 1 comprised of 25 neonates suffering from hypoxic ischemic encephalopathy of mild degree where as Group 2 comprised and base deficit >12mmol/l) Multiorgan involvement. Abnormal neurology or clinical seizures.Demographic data was collected on a proformaalong 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.Evidence of metabolic acidosis in umbilical arterial blood, or very early neonatal blood gas samples (pH <7 Anycondition 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. The collected data was entered into SPSS version 16.

Neonatal blood samples were obtained from arteries of neonate.Arterial blood gas analysis of blood was done from laboratory of Services hospital Lahore. Rest of the sample of blood was used for determination of glutamate and iron. Serum glutamate levels were measured by Enzyme linked immunosorbant assay (ELISA) using a commercially available kit (Biovision research products). Serum ferrous was measured by colorimetric method for iron determination without deproteinization using a commercially available kit (Wiener Lab).

RESULTS

The quantitative measures include glutamate and ferrous and presented as mean and standard deviation. The levels of glutamic acid and iron were compared within three groups of asphyxiated and non-asphyxiated neonates by applying ANOVA for significance. A *p* value of less than 0.05 was taken as significant. Comparison between the groups and within the groups was done by applying Post Hoc Test.





DISCUSSION

The relationship between glutamate and ferrous can be visualized using scatter gram in which a positive correlation of high magnitude has been found between glutamate and ferrous. The x –axis in this diagram is used to measure glutamate and the y-axis measure ferrous. The cluster is approaching a line in appearance, so the two characteristics are likely to be linearly correlated.

Similar to our study it was found in another study that NMDA receptor channels may contain a novel site sensitive to blockage by ferrous ion at their extracellular surfaces in immature cultured rat cortical neurons. This mechanism may be operative under particular condition associated with abnormalities of NMDA receptor channels in the brain[11]. It has been proved by Qureshi GA that nitric oxide and Glutamate potentiate the effects of each other. Based on the results of present study, we can say that neurotoxicity depends on generation of free radicals. Moreover free radicals and excitatory amino acids acts in concert to potentiate the effects of each other[12].

CONCLUSIONS

The significant elevation of serum glutamate in hypoxic neonates reiterates the key role of serum glutamate and ferrous in neonates suffering from hypoxic ischemic encephalopathy. The current study thus provides useful local data suggesting an association between elevated plasma glutamate and ferrous levels in patients with hypoxic ischemic encephalopathy. This study provides information that plasma glutamate and ferrous seems to act in their role of producing free radicals.

Our study highlights that further evaluation of the role of glutamate and ferrous as potential diagnostic target needs to be done due to its role in hypoxic ischemic progression. Additional studies are warranted to further clarify the oxidative stress by glutamate and ferrous. Further scientific exploits in this area need to be aimed at devising effective therapeutic agents which can antagonize the effects of ferrous and glutamate.

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