**ORIGINAL ARTICLE**

# Assessment of Glutamate Levels in Neonates Suffering from Hypoxic Ischemic Encephalopathy

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## ABSTRACT

**Aim:** To assess the glutamate level in neonates suffering from Hypoxic Ischemic Encephalopathy.
**Study design**: Descriptive analytic study
**Place and duration of study:** This study was conducted at the University of Health Sciences (UHS) Lahore in collaboration with Services Hospital Lahore.
**Methodology:** 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**: The level of serum glutamate was significantly higher in neonates suffering from hypoxic ischemic encephalopathy.
**Conclusion:** Elevated levels of serum glutamatesummarizes the role of glutamate in neonates suffering from hypoxic ischemic encephalopathy.
**Keywords**: Hypoxic ischemic encephalopathy, glutamate, oxidative stress

## INTRODUCTION

Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS). It participates in various brain functions such as memory and learning, development and aging and adaptation to the environment[1]. It is present in pre-synaptic nerve terminal from where it is released in synaptic cleft, and it activates postsynaptic receptors. Its receptors can be divided into two groups, ionotropic and metabotropic receptors.[2]these receptors mediate further calcium influx, voltage dependent block by magnesium, glycine co-activator, zinc inhibition and large single channel conduction[3]. They are agonist channels which open after activation by glutamate, $Na^{1+}$ and $Ca^{2+}$ ions entry into the cell. Their response is slower than that of non-NMDA receptors[4].

Excitotoxicity is a mechanism of neuronal injury which is involved in the pathogenesis of acute and chronic neurological disorders including hypoxic ischemia.[5]. Initially acute neuronal damage occur which is followed by reperfusion injury. However, during the secondary energy failure state decline in cerebral function occur[6]. Changes in amino acid neurotransmitter are evident after 4 days post-hypoxic ischemia (HI) in the new born piglet[7]. This energy depletion causes failure of $Na^+$ and $K^+$ pumps. As a result, sodium, calcium and water accumulate inside the cells causing a cytotoxic edema. It further decreases oxygen and glucose delivery to neurons, facilitating depolarization of cell membranes, opening up of calcium channels and depolarization of cell membranes[8]. Massive release of calcium occurs which causes excessive release of excitatory amino acids such as glutamate in the extracellular space[9]. It is cleared from the synaptic cleft through specific high affinity sodium dependent excitatory amino acid transporters. These transporters are modified by the redox state of the cell so higher oxidative stress aggravates cell damage[10].

Over activation of glutamate receptors leads to increased release of ions such as $Na^+$ and $Ca^{++}$ and $Cl^-$ creating iron overload.[11] 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[12]. NO, ROS and peroxynitrite are involved in the activation of apoptotic death pathway through caspase dependant pathway[13]. Glutamate mediated excitotoxicity can be mediated through apoptosis which is more important in immature brain. Bax is a member of Bcl-2 family of proteins. It is a strong pro-apoptotic protein in neuron.[14]Bax mediated cell death through the release of apoptogenic factor such as cytochrome c is found to play a key role in the promotion of apoptosis[16].

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## 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 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 whereas Group 2 comprised and base deficit >12 mmol/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 1 minute and at 5min less than 7. Evidence of metabolic acidosis in umbilical arterial blood, or very early 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. Rest of the sample of blood was used for determination of glutamate and iron. Serum glutamate levels were measured by Enzyme linked immunosorbent assay (ELISA) using a commercially available kit

## RESULTS

The collected data was entered into SPSS version 16.The quantitative measures include glutamate and ferrous and presented as mean and standard deviation. The level of glutamic acid was 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.

ANOVA: The correlation between serum glutamate levels in hypoxic and control was found to be significant (p= 0.000).

| Groups | Sum of squares | Mean square |
|---|---|---|
| Between Groups | 10874.885 | 5437.442 |
| Within Groups | 7120.931 | 87.913 |

P value: 0.000

**Post Hoc Tests:** On applying Post Hoc Tests mean difference between normal and HIE-1, normal and HIE 2&3 is significant and difference between HIE2 & 3 and HIE -1 is also significant. Differences of serum glutamate values are significant among all groups with a p value of less than 0.005.

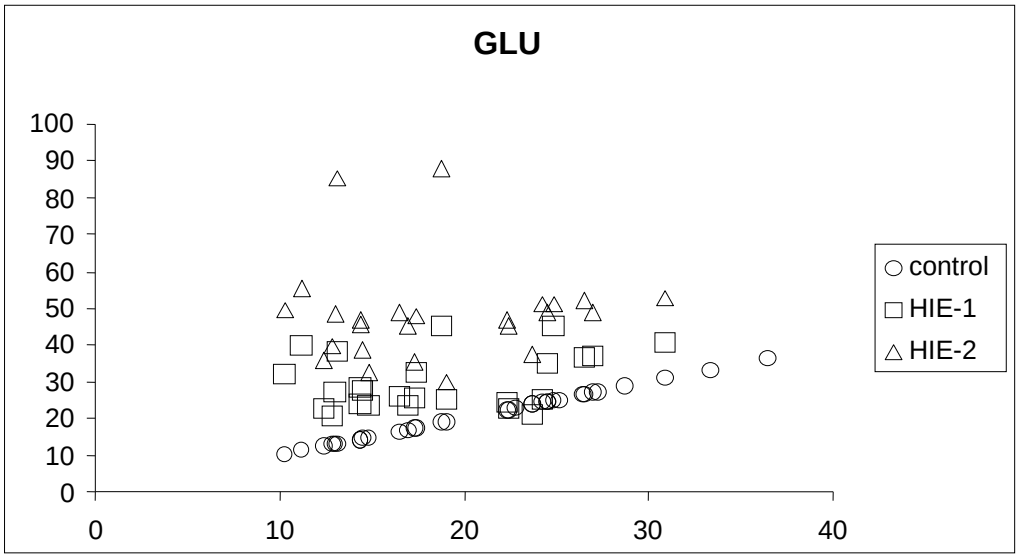


Table 8: Multiple Comparison of Glutamate between the groups

| (I)Groups | (J) Groups | Mean difference | Std Error | Significance |
|---|---|---|---|---|
| Normal | HIE-1<br>HIE 2&3 | -9.06437*<br>-27.33349* | 2.47026<br>2.47026 | .001<br>.000 |
| HIE-1 | Normal<br>HIE 2&3 | 9.06437*<br>-18.26912* | 2.47026<br>2.65198 | .001<br>.000 |
| HIE 2 & 3 | Normal<br>HIE-1 | 27.33349*<br>18.26912* | 2.47026<br>2.65198 | .000<br>.000 |

## DISCUSSION

There was a significant elevation of glutamate in hypoxic neonates as compared to control.Elevated glutamate levels indicate its association with oxidative stress in neonates. Mean values of glutamate in control neonates was 2.0934E1±1.152334 while that of neonates of HIE grade 1 2.9999E1±1.511365*** and of grade2-3 is 4.8268E1±2.664746***, showing a highly significant relationship between the degree of hypoxia and the concentration of neurotransmitter. Scatter graph of glutamate showed a positive correlation between glutamate and severity of hypoxic ischemic encephalopathy.

Our data is consistent with findings of Gucuyener, who showed that the high CSF levels of glutamatic acid and aspartic acid in CSF are correlated with the degree of hypoxic ischemic encephalopathy and the varying outcome[16].That study, however included cerebrospinal fluids of neonates suffering from hypoxic ischemic encephalopathy. Similar to our results Hagberg found cerebrospinal concentration of aspartate and glutamate higher in the groups with severe HIE than mild HIE.[17]Volpe reported that elevation in extracellular glutamate was responsible for causing toxicity to oligodendrocytes (OL) precursors by both receptors mediated and non-receptor mediated mechanisms thereby suggesting pathogenesis of periventricular leukomalacia (PVL)[18].

## CONCLUSIONS

The elevated levels of serum glutamate recapitulate the role of serum glutamate in neonates suffering from hypoxic ischemic encephalopathy. This study provides data that serum glutamate plays role in generation of free radicals. Further scientific exploits in this area needs to be aimed at devising effective therapeutic agents which can antagonize the effects of glutamate.

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