STUDY OF INTERLEUKIN-6 (IL-6) LEVELS IN PERINATAL ASPHYXIA AND ITS CORRELATION WITH DIFFERENT STAGES OF HYPOXIC ISCHEMIC ENCEPHALOPATHY

Dr. Sudhir Mehta*

Department of Pediatrics, Neonatology Section, Bhandari Hospital and Research Center, Indore, Madhya Pradesh, India.

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ABSTRACT

Introduction: Perinatal asphyxia is a major cause of neurologic morbidity in infants. Hypoxic ischemic encephalopathy (HIE) after perinatal asphyxia is a condition in which serum concentration of brain specific biochemical markers may be elevated. Neuroprotective interventions in asphyxiated newborns require early indicators of brain damage to initiate therapy. There are very few studies about its usefulness in asphyxiated newborns.

Aims and Objectives: To determine the serum levels of interleukin-6 (IL-6) in newborns with perinatal asphyxia and its relation with different stages of HIE.

Methods: We have measured the serum values of IL-6 by enzyme linked immunosorbant assay method in 80 asphyxiated newborns and 80 healthy newborns (control group). Blood samples were taken on day 1 and day 3 of life in all newborns.

Results: The mean serum values of IL-6 were found to be decreased on day 3 in asphyxiated neonates and a negative correlation was seen between day 1 and 3 for IL-6. The mean values of IL-6 were decreased in different stages of HIE on day 3 as compared to day 1 and a negative correlation was observed between day 1 and day 3 for IL-6 in no HIE, HIE I, HIE II and HIE III stages.

Conclusion: We conclude that serum IL-6 concentrations increased considerably after birth asphyxia, and the increase is associated with the severity of HIE with a poorer outcome.

KEYWORDS: perinatal asphyxia, Hypoxic-ischemic encephalopathy, Interleukin-6.
INTRODUCTION
Perinatal asphyxia is a common cause of neonatal morbidity and mortality in the neonatal period and long-term neurologic disabilities among survivors. Hypoxic-ischemic encephalopathy (HIE) of the newborn occurs with the incidence of 1-4/1000. Between 20% and 50% of newborn infants affected by perinatal brain injury die during the newborn period, and 25-60% of the survivors suffer from permanent neurodevelopment handicaps, including cerebral palsy, seizures, mental retardation, and learning disabilities. Four million neonates are affected by severe perinatal asphyxia worldwide each year. Out of these, 800,000 die as a result, and another 800,000 develop clinically significant sequelae.

There is evidence supporting the involvement of the inflammatory cascade in the pathogenesis of ischemic brain injury. Inflammation triggered by ischemia of the central nervous system (CNS) is characterized by polymorph nuclear cell recruitment, requiring the expression of specific adhesion molecules and chemotactic factors, and is followed by monocytes and microglial activation. Interleukins are synthesized and secreted in response to stimuli by lymphocytes, monocytes, and macrophages. Mild encephalopathy carries a good prognosis, although, in moderate and severe encephalopathy, the risk of death or neurologic sequelae increases to a great extent. Various indicators of brain damage have been investigated in the last decade.

For justifying the administration of certain drugs and management of asphyxiated neonates, early recognition of HIE is important. There are various experimental studies which suggest that cytokine mediated inflammatory reactions are important in the cascade that lead to hypoxic-ischemic brain injury. IL-6 is a cytokine that provokes a broad range of cellular and physiological responses, including the immune response, inflammation, haematopoiesis and oncogenesis by regulating cell growth, gene activation, proliferation, survival, and differentiation but still its role as a potential mediator during the progression of brain injury is unclear. To the best of author’s knowledge, very few previous studies are available regarding this brain specific biochemical marker in asphyxiated newborns. In the present study, we investigated the serum levels of IL-6 in asphyxiated newborns and their relation with different stages of HIE.

METHODOLOGY
The study was undertaken at Bhandari hospital and research center Indore, which is a tertiary care hospital. The study included 80 asphyxiated newborns as the study group and 80 healthy
newborns as a control group.

**Inclusion Criteria**
The newborns admitted in the Department of Pediatrics, and its neonatal unit were enrolled for the present study. Gestational age, birth weight, relevant perinatal history, findings on physical examination and systemic signs were recorded on a predesigned pretested proforma in both the groups. The study group was further divided according to Sarnat and Sarnat classification as No HIE group, mild HIE (Grade I), moderate HIE (Grade II) and severe HIE (Grade III).

**Exclusion Criteria**
Predefined exclusion criteria for both the groups were congenital anomalies, maternal drug addiction, neonatal sepsis and inborn error of metabolism.

**Blood Sampling and Analysis**
Blood samples (2 ml) were collected on day 1 and day 3 neonatal of life. Serum was carefully separated by centrifugation and then stored in aliquots at −80°C until analysis. IL-6 levels were measured by solid enzyme linked immunosorbent assay (ELISA), solid phase sandwich ELISA.

**Statistical Analysis**
The present study was a case control study. For statistical analysis, we used SPSS Software version 16 (IBM Corp). For comparison between cases and control group, we used statistical tools-descriptive statistics, unpaired t-test and paired t-test. Correlation was calculated by Pearson’s correlation coefficient. Confidence interval was calculated using software STATA (Stata corp LP).

**RESULTS**
Total 80 asphyxiated newborns and 80 healthy newborns were included in the study. The mean gestational age of cases is 37.35 ± 2.13 and of controls is 37.44 ± 2.12. The mean birth weight in cases and controls were 2.58 ± 0.60 and 2.67 ± 0.52 respectively. Number of male/female in the cases and controls were 44/36 and 39/41 respectively. In our study number of babies delivered by vaginal/caesarean lower segment caesarean section in cases and controls were 48/52 and 32/28 respectively (Table 1). Of the 80 cases, 2 asphyxiated newborns expired on day 5.
Out 80 asphyxiated newborns, 15 had no HIE, 25 developed HIE Grade I, 28 Grade II, and 12 Grade III. The concentrations of serum IL-6 on the 1st day were statistically significantly higher in the asphyxiated group compared with the control group ($P < 0.001$).

Serum IL-6 concentrations in asphyxiated neonates on the 1st day was $88.36 \pm 47.46$ pg/mL while on day 3 was, $74.66 \pm 61.96$ pg/mL (Table 2).

Among the infants in whom HIE developed, 1st day serum IL-6 levels were $27.98 \pm 29.32$ pg/ml in HIE Stage 0 (no HIE), $58.98 \pm 42.09$ pg/mL in those with Stage 1 HIE, $89.53 \pm 59.71$ pg/mL with Stage II HIE and $157.90 \pm 51.65$ pg/mL with Stage III HIE. On day three, the mean serum values of IL-6 in Stage 0 (no HIE), HIE I, HIE II and HIE III were $19.57 \pm 13.83$ pg/mL, $49.89 \pm 45.83$ pg/mL, $69.69 \pm 48.17$ pg/mL and $129.78 \pm 49.70$ pg/mL respectively (Table 3). The mean values of IL-6 were decreased in different stages of HIE on day 3 as compared to day 1 in asphyxiated neonates.

### Table 1: Demographic profile of study group (cases) and controls

<table>
<thead>
<tr>
<th>Demographic variables</th>
<th>Cases</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of newborns</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>37.35 ± 2.13</td>
<td>37.44±2.12</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.58 ± 0.60</td>
<td>2.67 ± 0.52</td>
</tr>
<tr>
<td>Male/female</td>
<td>44/36</td>
<td>39/41</td>
</tr>
<tr>
<td>Number of vaginal deliveries</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>Number of LSCS deliveries</td>
<td>52</td>
<td>28</td>
</tr>
</tbody>
</table>

### Table 2: Comparison of mean values of IL-6 on day 1 and day 3 in cases of birth asphyxia and their correlation

<table>
<thead>
<tr>
<th>IL-6 (pg/mL)</th>
<th>Cases</th>
<th>Mean±SD</th>
<th>$r$ value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>100</td>
<td>88.36 ±47.46</td>
<td>−0.773**</td>
<td>0.000</td>
</tr>
<tr>
<td>Day 3</td>
<td>98</td>
<td>74.66 ±61.96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Comparison of mean values of IL-6 on day 1 and day 3 in different stages of HIE and their correlation

<table>
<thead>
<tr>
<th>Stages of HIE</th>
<th>Mean±SD</th>
<th>$r$ value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Day 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No HIE (0)</td>
<td>27.98±29.32</td>
<td>19.57±13.83</td>
<td>−0.533**</td>
</tr>
<tr>
<td>I</td>
<td>58.98±42.09</td>
<td>49.89±45.83</td>
<td>−0.850**</td>
</tr>
<tr>
<td>II</td>
<td>89.53±59.71</td>
<td>69.69±48.17</td>
<td>−0.894**</td>
</tr>
<tr>
<td>III</td>
<td>157.90±51.65</td>
<td>129.78±48.70</td>
<td>−0.887**</td>
</tr>
</tbody>
</table>
DISCUSSION
In the present study, we have determined serum levels of IL-6 in asphyxiated and healthy neonates. Serum IL-6 concentrations in the first day of life were significantly elevated in cases compared with the healthy controls, and these elevated concentrations were associated with the severity of asphyxia and a poorer outcome. The elevated serum IL-6 levels could be indicative of the involvement of this cytokine as a potential mediator of asphyxia. Previously, elevated levels of serum IL-1β, IL-8 and IL-6 have been reported for infants at term. Similar to these findings, the concentrations of serum IL-6 have been reported to be higher in asphyxiated neonates than those of normal neonates.\textsuperscript{[15]} However, we have studied a larger population and prospectively measured serum IL-6 concentrations in asphyxiated neonates and age-matched healthy controls at fixed time points postpartum i.e., in the first and third day of life.

Various diagnostic modalities are available to diagnose neonatal brain injury in perinatal asphyxia. Neuronal necrosis and apoptosis after ischemic episode are slow and also lasts for several hours to several days as compared to studies conducted in perinatal animals that suggest a quicker cellular destruction. Energy substrates begin to decrease for 12-48 h after hypoxia in the neonatal brain. There are various neuroprotective interventions, but these may be harmful, so it is very important to find early and reliable indicators of brain damage or of poor long-term prognosis to initiate or end neuroprotective treatment. Cranial tomography, somatosensory evoked potentials, and magnetic resonance tomography are useful for prognosis, but not in the first 24 h after birth. IL-6 appears as an early marker of hypoxic ischemic brain injury.\textsuperscript{[16]} The rise in serum IL-6 response within the first 24 h after hypoxic ischemic insult provides an additional support for the possible role of IL-6 in the pathogenesis of brain injury. There are possibilities that IL-6 might be released as a protective response after hypoxic ischemic brain injury, and might be involved in the repair mechanisms in the sub-acute stage of HIE.\textsuperscript{[17]}

In this study, IL-6 concentrations decreased on day 3. IL-6 is a pleiotropic cytokine having proinflammatory and anti-inflammatory potentials.\textsuperscript{[17]} To understand the bimodal action of IL-6 functions in the pathogenesis of HIE, further studies are required.\textsuperscript{[18]}

The present study determined serum levels of IL-6 in asphyxiated and healthy newborns. Serum IL-6 concentrations in the 1\textsuperscript{st} day of life were significantly elevated in cases compared with the healthy controls, and these elevated concentrations were associated with the severity
of asphyxia.

The elevated serum IL-6 levels may indicate the involvement of this cytokine as a potential mediator of asphyxia. Earlier, elevated levels of serum IL-1, IL-8 and IL-6 have been reported for term infants.\cite{19,20} We have studied a larger population and measured serum IL-6 concentrations in asphyxiated newborns and healthy controls on the 1\textsuperscript{st} and 3\textsuperscript{rd} day of life. Similar to these results, the concentrations of serum IL-6 have been reported to be higher in asphyxiated newborns than those of normal newborns.\cite{1,20}

Regarding the newborns who were diagnosed with HIE, a significant association was observed between serum IL-6 concentrations and Sarnat’s grading of the severity of encephalopathy. These results are also concordant with the finding by Aly et al.\cite{21} who have reported that serum IL-6 concentrations were significantly correlated to the Sarnat’s grading of encephalopathy.

**CONCLUSION**

We conclude that serum IL-6 concentrations increased considerably after birth asphyxia, and these increases were associated with the severity of encephalopathy and a poorer outcome. Hence IL-6 might have an important role following injury to the CNS, and serum concentrations appear to be a good predictor of outcome in HIE. However, more investigations are required for better understanding of the role of this cytokine in cerebral injury caused by hypoxic insult.

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**REFERENCES**


