Original Article

Study of Neurological Marker in Perinatal Asphyxia and Its Correlation with Different Stages of Hypoxic Ischemic Encephalopathy

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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 100 asphyxiated newborns and 100 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: Enzyme linked immunosorbant assay, 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.[1] Hypoxic-ischemic encephalopathy (HIE) of the newborn occurs with the incidence of 1-4/1000.[2] 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.[3,4]

Mild encephalopathy carries a good prognosis, although, in moderate and severe encephalopathy, the risk of death or neurologic sequelae increases to a great extent.[5] Various indicators of brain damage have been investigated in the last decade.[6-12] 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. Interleukins (ILs) are synthesized and secreted

in response to stimuli by lymphocytes, monocytes, and macrophages.

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 our 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 with the approval of Institutional Ethical Committee of the Medical Faculty of S.A.I.M.S. Medical College and PG Institute, Indore, Madhya Pradesh.

The study included 100 asphyxiated newborns as the study group and 100 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, tumors, maternal drug addiction, severe infections and congenital mental disorders.

Blood Sampling and Analysis

Blood samples (1-2 ml) were collected on day 1 and day 3 of life. Serum was carefully separated by centrifugation and then stored in aliquots at −70°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, and the method of sampling used was non-random-purposive. For statistical analysis, we used SPSS Software version 16 (IBM Corp). For comparison between cases and control group, we used statistical tools-descriptive statistics, diagrammatic representation, unpaired *t*-test and paired *t*-test. Correlation was calculated by Pearson's correlation coefficient (two-tailed). Confidence interval was calculated using software STATA (Stata corp LP).

RESULTS

Total 100 asphyxiated newborns and 100 healthy newborns were included in the study. The mean gestational age of cases is 38.02 ± 2.53 and of controls is 38.44 ± 2.22. The mean birth weight in cases and controls were 2.68 ± 0.69 and 2.77 ± 0.54 respectively. Number of male/female in the cases and controls were 67/33 and 54/46 respectively. In our study number of babies delivered by vaginal/caesarean lower segment caesarean section in cases and controls were 58/60 and 42/40 respectively (Table 1). Of the 100 cases, 2 asphyxiated newborns expired on day 3.

Out 100 asphyxiated newborns, 18 had no HIE, 20 developed HIE Grade I, 41 Grade II, and 21 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 78.36 ± 57.46 pg/mL while on day 3 was, 64.66 ± 51.96 pg/mL (Table 2).

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

Demographic variables	Cases	Control
Number of newborns	100	100
Gestational age (weeks)	38.02±2.53	38.44±2.22
Birth weight (kg)	2.68±0.69	2.77±0.54
Male/female	67/33	54/46
Number of vaginal deliveries	58	60
Number of LSCS deliveries	42	40

LSCS: Lower segment caesarean section

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

IL-6 (pg/mL)	Cases	Mean±SD	*r* value	*P* value
Day 1	100	76.41±56.37	−0.973**	0.000
Day 3	98	64.66±51.96		

IL: Interleukin, SD: Standard deviation, **statistically highly significant

Among the infants in whom HIE developed, 1st day serum IL-6 levels were 27.98 ± 29.32 pg/ml in HIE Stage 0 (no HIE), 58.98 ± 52.09 pg/mL in those with Stage 1 HIE, 79.53 ± 49.71 pg/mL with Stage II HIE and 137.70 ± 41.65 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 17.42 ± 12.83 pg/mL, 46.99 ± 47.83 pg/mL, 66.69 ± 44.17 pg/mL and 120.78 ± 39.70 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.

DISCUSSION

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.[13] 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.[14]

In our study, IL-6 concentrations decreased on day 3. IL-6 is a pleiotropic cytokine having proinflammatory and anti-inflammatory potentials.[14] To understand the bimodal action of IL-6 functions in the pathogenesis of HIE, further studies are required.[15]

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

Stages of HIE	Mean±SD		*r* value	*P* value
	Day 1	Day 3		
No HIE (0) (n=18)	27.98±29.32	17.42±12.83	−0.633**	0.005
I (n=20)	58.98±52.09	46.99±47.83	−0.950**	0.000
II (n_1=41) (n_3=40)	79.53±49.71	66.69±44.17	−0.994**	0.000
III (n_1=21) (n_3=20)	137.70±41.65	120.78±39.70	−0.987**	0.000

IL: Interleukin, SD: Standard deviation, HIE: Hypoxic ischemic encephalopathy, **statistically highly significant

In the present study, we have determined serum levels of IL-6 in asphyxiated and healthy newborns. Serum IL-6 concentrations in the 1st 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.[16,17] We have studied a larger population and measured serum IL-6 concentrations in asphyxiated newborns and healthy controls on the 1st and 3rd day of life. Similar to our results, the concentrations of serum IL-6 have been reported to be higher in asphyxiated newborns than those of normal newborns.[1,17]

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. Our results are also concordant with the finding by Aly *et al.*[18] 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 the increase is associated with the severity of HIE with a poorer outcome. Hence, IL-6 might have an important role following injury to the central nervous system, and serum concentrations appear to be a good predictor of outcome in HIE.

However, more investigations and further studies are required for better understanding of the role of IL6 after hypoxic ischemic brain injury.

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