ejpmr, 2015,2(3), 862-869

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

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.

Article Received on 06/04/2015 **Article Revised on 01/05/2015** **Article Accepted on 26/05/2015**

***Correspondence for Author**

Dr. Sudhir Mehta

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

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 relationwith different stages of HIE. **Methods:** We have measured the serum values of IL-6 by enzyme linked immunosorbant assay method in 80 asphyxiatednewborns 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 negativecorrelation 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 isassociated 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.[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] 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.[5]

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.[6,7] Interleukins are synthesized and secreted in response to stimuli by lymphocytes, monocytes, and macrophages.[6] Mild encephalopathy carries a good prognosis, although, in moderate and severe encephalopathy, the risk of death or neurologic sequelae increases to a great extent.[8] Various indicators of brain damage have been investigated in the last decade.[9-15]

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-descriptivestatistics, unpaired *t*-test and paired *t*-test. Correlation was calculated by Pearson's correlationcoefficient. 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 ± 47.46 pg/mL while on day 3 was, 74.66 ± 61.96 pg/mL (Table 2).

Among the infants in whom HIE developed, 1 st day serum IL-6 levels were 27.98 ± 29.32 pg/ml in HIE Stage 0 (no HIE), 58.98 ± 42.09 pg/mL in those with Stage 1 HIE, 89.53 ± 59.71 pg/mL with Stage II HIE and 157.90 ± 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 ± 13.83 pg/mL, 49.89 ± 45.83 pg/mL, 69.69 ± 48.17 pg/mL and 129.78 ± 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

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

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	88.36 ±47.46	−0.773**	0.000
Day 3	98	74.66 ±61.96		

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)	27.98±29.32	19.57±13.83	−0.533**	0.004
I	58.98±42.09	49.89±45.83	−0.850**	0.000
II	89.53±59.71	69.69±48.17	−0.894**	0.000
III	157.90±51.65	129.78±48.70	−0.887**	0.000

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.[15] However, we have studied a larger population and prospectively measured serum IL-6 concentrations in asphyxiated neonates and age-matched healthy controls at t 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.[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.[17]

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

The present study 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.[19,20] 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 these results, the concentrations of serum IL-6 have been reported to be higher in asphyxiated newborns than those of normal newborns.[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.[21] who have reported that serum IL-6 concent rations were significantly correlated to the Sarnat's grading of encephalopathy.

CONCLUSION

We conclude that serum IL-6concentrations increased considerably after birthasphyxia, and these increases were associatedwith the severity of encephalopathy and a pooreroutcome. Hence IL-6 might have an important rolefollowing injury to the CNS, and serum concentrationsappear to be a good predictor of outcome in HIE.However, more investigations are required for betterunderstanding of the role of this cytokine in cerebralinjury caused by hypoxic insult.

ACKNOWLEDGEMENT: none

FUNDING: none

CONFLICT OF INTERESTS: none

REFERENCES

1. Chiesa C, Pellegrini G, Panero A, De Luca T, Assumma M, Signore F, et al. Umbilical cord interleukin-6 levels are elevated in term neonates with perinatal asphyxia. Eur J Clin Invest., 2003; 33: 352-8.
2. Vannucci RC. Hypoxic-ischemic encephalopathy. Am J Perinatol., 2000; 17: 113-20.
3. Derganc M, Osredkar D. Hypoxic-ischemic brain injury in the neonatal period. ZdravVestn., 2008; 77: 51-8.

4. Miller SP, Ramaswamy V, Michelson D, Barkovich AJ, Holshouser B, Wycliffe N, et al. Patterns of brain injury in term neonatal encephalopathy. J Pediatr., 2005; 146: 453-60.
5. De Costello LAM, Manandhar DS.Perinatal asphyxiain less developed countries. Arch Dis Child FetalNeonatal Ed., 1994; 71: F1-3.
6. Ceccon ME. Interleukins in hypoxic-ischemicencephalopathy.J Pediatr (Rio J)., 2003; 79: 280-1
7. Silveira RC, Procianoy RS. Interleukin-6 and tumonecrosis factor-alpha levels in plasma and cerebrospinal fluid of term newborn infants with hypoxic-ischemic encephalopathy.J Pediatr., 2003; 143: 625-9.
8. Finer NN, Robertson CM, Richards RT, Pinnell LE, Peters KL. Hypoxic-ischemic encephalopathy in term neonates: Perinatal factors and outcome. J Pediatr., 1981; 98: 112-7.
9. Hellström-Westas L, Rosén I, Svenningsen NW. Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. Arch Dis Child Fetal Neonatal Ed., 1995; 72: F34-8.
10. al Naqeeb N, Edwards AD, Cowan FM, Azzopardi D. Assessment of neonatal encephalopathy by amplitude-integrated electroencephalography. Pediatrics., 1999; 103: 1263-71.
11. Thoresen M, Penrice J, Lorek A, Cady EB, Wylezinska M, Kirkbride V, et al. Mild hypothermia after severe transient hypoxia-ischemia ameliorates delayed cerebral energy failure in the newborn piglet. Pediatr Res., 1995; 37: 667-70.
12. Blennow M, Hagberg H, Rosengren L. Glial fibrillary acidic protein in the cerebrospinal fluid: A possible indicator of prognosis in full-term asphyxiated newborn infants? Pediatr Res., 1995; 37: 260-4.
13. Hagberg H, Thornberg E, Blennow M, Kjellmer I, Lagercrantz H, Thiringer K, et al. Excitatory amino acids in the cerebrospinal fluid of asphyxiated infants: Relationship to hypoxic-ischemic encephalopathy. ActaPaediatr., 1993; 82: 925-9.
14. Martín-Ancel A, García-Alix A, Pascual-Salcedo D, Cabañas F, Valcarce M, Quero J. Interleukin-6 in the cerebrospinal fluid after perinatalasphyxia is related to early and late neurological manifestations. Pediatrics., 1997; 100: 789-94.
15. Huang CC, Wang ST, Chang YC, Lin KP, Wu PL. Measurement of the urinary lactate: creatinine ratio for the early identification of newborn infants at risk for hypoxic-ischemic encephalopathy. N Engl J Med., 1999; 341: 328-35.

16. Selmaj KW, Farooq M, Norton WT, Raine CS, Brosnan CF. Proliferation of astrocytes in vitro in response to cytokines. A primary role for tumor necrosis factor. J Immunol,m 1990; 144: 129-35.
17. Hassan B, Afsharib JT, Mobarhanc MG, Maamouria GD, Shakerie MT, Sahebkarc A, et al. Association between serum interleukin-6 levels and severity of perinatal asphyxia. Asian Biomed, 2010; 4: 79-85.
18. Tekgul H, Yalaz M, Kutukculer N, Ozbek S, Kose T, Akisu M, et al. Value of biochemical markers for outcome in term infants with asphyxia. PediatrNeurol, 2004; 31: 326-32.
19. Shalak LF, Laptook AR, Jafri HS, Ramilo O, Perlman JM. Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. Pediatrics, 2002; 110: 673-80.
20. Xanthou M, Fotopoulos S, Mouchtouri A, Lipsou N, Zika I, Sarafidou J. Inflammatory mediators in perinatal asphyxia and infection. ActaPaediatrSuppl., 2002; 91: 92-7.
21. Aly H, Khashaba MT, El-Ayouty M, El-Sayed O, Hasanein BM. IL-1beta, IL-6 and TNF-alpha and outcomes of neonatal hypoxic ischemic encephalopathy. Brain Dev., 2006; 28: 178-82.