Association between serum interleukin-1β levels and perinatal asphyxia

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Abstract

Objective:
Asphyxia is a major cause of acute mortality and chronic neurologic disability in neonates. We sought to define the predictive values of serum concentrations of interleukin-1β in newborns with perinatal asphyxia to see if there is a relation between interleukin-1β (IL-1β) levels to the short term neurological deficit.

Methods:
This was a prospective (case-control) study conducted between June 2007 and July 2008, at the Neonatal Intensive Care Unit, Ghaem Hospital, Mashhad, Iran. Serum IL-1β levels were measured at birth, 24 and 48 h post-partum in 38 consecutive uninfected neonates with perinatal asphyxia(blood pH< 7.2, low Apgar score , signs of fetal distress) and 41 randomly selected healthy newborns (normal infants free of a postnatal clinical event during the first weeks of life). Receiver-operating characteristic (ROC) curves were used for the determination of thresholds for the asphyxiated group versus healthy neonate group.

Results:
A total of 79 infants were studied. Serum interleukin-1β concentrations in the infants who developed hypoxic-ischemic encephalopathy was 6 folds higher as compared to values in the normal infants (p< 0.006) and 5-folds higher compared to infants with asphyxia who did not subsequently develop hypoxic-ischemic encephalopathy (p< 0.006). There was also a significant relationship between serum IL-1B and outcome at the time of discharge.

Conclusions:
Serum levels of IL-1B are increased substantially in neonates with asphyxia, and this is most pronounced in neonates with poorer prognosis.

Keywords:
Hypoxic- ischemic encephalopathy, Interleukin-1β, Newborn, Perinatal asphyxia

Introduction

Four million children are born annually with severe perinatal asphyxia worldwide (1). Estimates suggest that between 2 and 4/1000 full-term newborn infants suffer From asphyxia at or shortly before birth. Approximately 15% to 33% of such asphyxiated infants who exhibit hypoxic-ischemic encephalopathy (HIE) actually die during the neonatal period. Of the survivors, 25% will exhibit permanent neuropsychological deficits(2, 3). There is evidence supporting the involvement of the inflammatory cascade in the pathogenesis of ischemic brain injury. IL-1β is an important cytokine released mainly by mononuclear cells and macrophages in response to infection

and tissue injury. The role of this cytokine in tissue injury due to perinatal asphyxia has also been investigated in experimental studies. (4, 5) However, it is not clear whether they have the same effect in human neonatal brain after perinatal asphyxia and whether they can accurately predict prognosis. Therefore we sought to define the predictive values of serum concentrations of interleukin-1β in newborns with perinatal asphyxia and to examine the relation of interleukin-1β (IL-1β) levels to the short term neurological outcome and severity of perinatal asphyxia.

Methods

This was a prospective (case control) study conducted between June 2007 and December 2008, at Ghaem Hospital in Mashhad, Iran. Parental informed consent was obtained before inclusion in the study. This study was undertaken with the approval of the Ethical Committee of Mashhad University of Medical Sciences.

Forty-seven cases were eligible for this study. Nine subjects were excluded for reasons related to problems with specimen collection, or clinical features, including insufficient blood sample (n= 2), congenital or perinatal infection (n= 2), histological or clinical chorioamnionitis, high maternal temperature during labor greater than 38 ℃, foul- smelling amniotic fluid (n= 3) and congenital malformation (n= 2).

Perinatal asphyxia was defined as the presence of at least two of the following conditions:

1) Signs of fetal distress (heart rate less than 100 beats per minute, late decelerations, or an absence of heart-rate variability)

2) Thick, meconium-stained amniotic fluid and respiratory depression hypotonia or bradycardia

3) Apgar score of 4 or less at 1 min or 6 or less at 5 min

4) A need for resuscitation for more than 1 min with positive_ pressure ventilation and oxygen immediately after birth

5) Blood pH value of less than 7.2 or a base deficit of at least 12 mmol per liter within the first hour after birth.

Forty-six healthy neonates (neonates who were free of postnatal clinical event during the first week of life) were recruited as controls. Among the control group, 5 infants were excluded due to incomplete data.

Newborn neurological examinations were performed by a single neonatologist experienced in neurological evaluation without knowledge of the cytokine concentration. An examination was used to evaluate neurological function of the neonate at birth, and on the 2nd and 7th days of life. This included a systematic assessment of mental status (level of alertness), cranial nerve function and the motor and sensory systems. In particular, the motor examination included an assessment of spontaneous movement and muscle tone. Posture and resistance of muscles to passive movement were used to assess active tone. The infants with perinatal asphyxia were divided according to whether or not HIE developed within the first 7 days after birth. According to the criteria of Sarnat HIE was classified as mild (Grade 1) if hyperexcitability or hyperalertness or hyper-reflexia persisted without seizures for at least 24 hr after birth; as moderate if the infant was lethargic, had hypotonia, weak primitive reflexes, pupil miosis and seizures; and as severe if the infant had apnea, flaccid weakness, frequent seizures, decelerated posture or coma.

In addition to neurologic dysfunction arising from HIE, endpoints of systemic complications within the first week of life included pulmonary ventilator dependence or the need for supplemental oxygen for> 24 hr, cardiovascular, congestive heart failure not associated with structural heart disease, or shock; gastrointestinal, gut ischemia; elevated hepatic transaminases, prolonged prothrombin time or partial thromboplastin time; thrombocytopenia, acute tubular necrosis, oliguria (<1 ml/ kg/hour urine flow rates) beyond 24 hr. The outcome was classified as favorable or adverse. A favorable outcome was defined as normal neurologic and good general condition at the end of the first month. Adverse outcome was defined as the presence of at least one of the following conditions: Hemiplegia, hypertonocity or significant hypotonia, insufficient sucking, seizures resistant to phenobarbital and sensory neural hearing loss.

Blood sample for case (n= 38) and control (n= 41) subjects were collected on the first, second and third days of life.

Serum IL-1β was measured on the first, second and third days of life. From each neonate with suspected asphyxia, a blood sample of

1-2 ml was taken by venipuncture for IL-1β determination. A similar blood sample was also obtained from umbilical cord or peripheral blood of 45 healthy neonates. Plasma was separated by centrifugation and then stored in aliquots at–70 ℃ until analysis. IL-1β levels were measured using a highly-sensitive and specific enzyme-linked immunosorbent assay kit (Bender Med system–GMOH). The minimum detectable concentration for IL-1β was 0.1 pg/ml. All samples were run in duplicate. Blood culture, cerebrospinal fluid culture, urine culture, serum creatinine, Na, K, calcium and IL-1β were determined at the time of the initial evaluations.

Descriptive statistics and analytical tests (Mann-Whitney rank–sum test, the Student t-test, chi-square test, and Spearman correlation coefficient) were performed by using SPSS software. Statistical comparisons between the groups (Asphyxiated group, HIE group and control group) were performed using ANOVA tests for continuous variables. Sensitivity and 95% confidence interval (CI) values were calculated for IL-1β. Receiver-operating characteristic (ROC) curves were determined for the neonatal group. The normality of quantitative variables was checked using one sample Kolmogorov- Smirnov test. Data was presented as mean±SD of both groups. p-value of < 0.05 was considered statistically significant.

Results

Seventy-nine neonates were studied [cases (n= 38) and controls (n= 41)]. There was no statistically significant difference (p< 0.05) between the two groups regarding age, gender, gestational age and maternal age (Table1).

In comparison with the controls, the cases had a significantly lower Apgar scores in the first minute and first five minutes post partum, higher length of hospital stay, higher likelihood of Cesarean section, complications during pregnancy or delivery and respiratory problems (p< 0.001) Tables 1and 2).

Of the 38 infants with perinatal asphyxia, 3 infants had no HIE, 11 had HIE grade1, 10 had grade 2, 8 had grade 3.

All infants in the asphyxia group had negative body-fluid cultures, and received antibiotic treatment for 5 days or less.

The concentrations of serum first-day IL-1β were considerably higher in the asphyxia group (p< 0.001) compared with the control group [18.35 (2.1-203) pg/ml vs. 3.69 (0-27 pg/ml)] The concentrations of serum first day IL-1β were 3.70 pg/ml for controls, 4.06 pg/ml for asphyxiated neonates without HIE, 11.78 pg/ml for HIE grade 1, 16.73 pg/ml for HIE grade 2, and 36.46 pg/ml for HIE grade 3(p= 0.012).

Serum IL-1β concentration on the first day was 20.30 pg/ml in the infants who subsequently developed HIE; this was 5 folds higher than for the normal infants (3.87 pg ML, p< 0.006).

Among the 32 infants who had HIE, 17 had favorable outcomes (neurologic development was normal), and 7 had adverse outcomes (neurodevelopment sequel). Median serum IL-1β concentrations were significantly higher in neonates with adverse outcomes than in those with favorable outcomes (42.82 pg / l vs.5.00 pg ml, p< 0.001, Figure 3).

An IL-1β concentration greater than 4 pg /dl had

Table -1. Clinical characteristics of the population studied

Group	Asphyxia	Control	P Value
Number	38	41	
Age(day)	1.1±3.1	1.0±0.2	>0.60
Birth weight(gr)	2799 ± 794	3299±507	<0.05
Gestational age(week)	38.05 ± 1.0	39.3 ± 1.3	>0.05
1-minute Apgar	4.6 ±1.7	8.6±0. 7	<0.001
5-minute Apgar	6.2±1.4	9±0.4	<0.001
Mode of dlivery(ND/CS)	13/25	27/13	<0.01
Duration hospital stay	7.5±5	0.4±1.4	<0.001
Maternal age	26±6.0	27± 5.2	>0.500
Sex(Male/Female)	15/23	21/20	>0.35

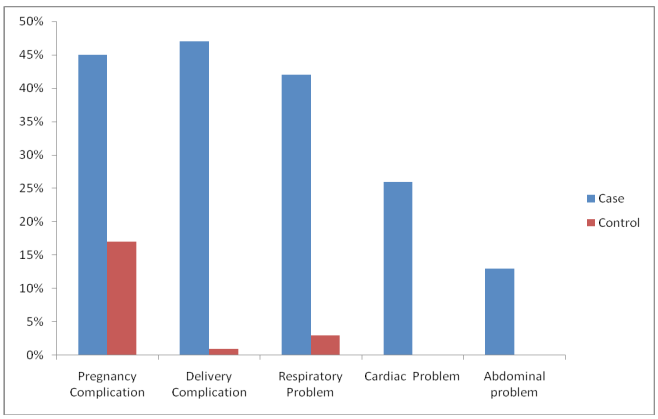


Figure -1. Comparison Risk factors and complication between Case and control groups

Pregnancy complication:eclampsia, pre-eclampsia, polyhydroaminus, epilepsy
Delivery complication:sufrance, placental apruption, placenta previa
Respiratory problem:tachypnoea,Apnoea,granting
Cardiac problem:cardiac murmur, bradycardia,cardiomegaly
Abdomen problem:feeding intolerance,abdominal distention

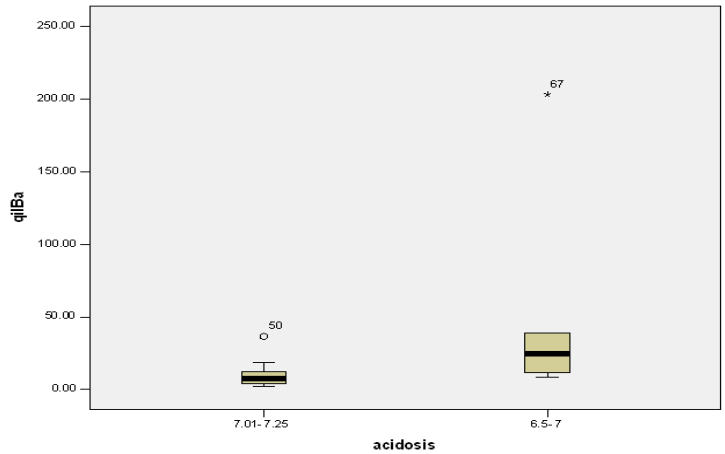


Figure -2 . Association among first day of IL-1β and severity of acidosis

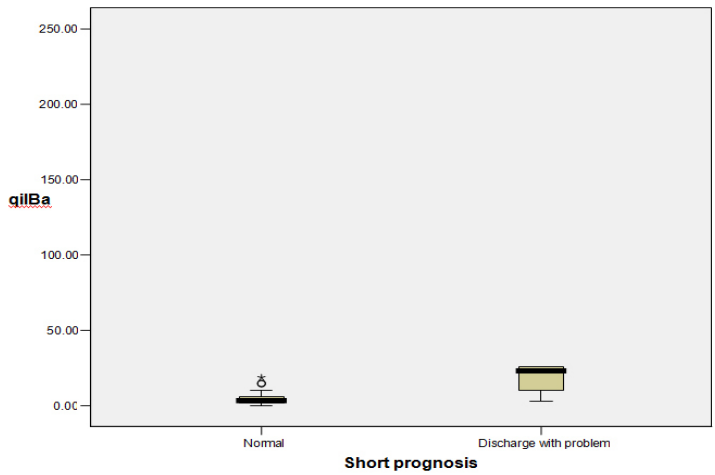
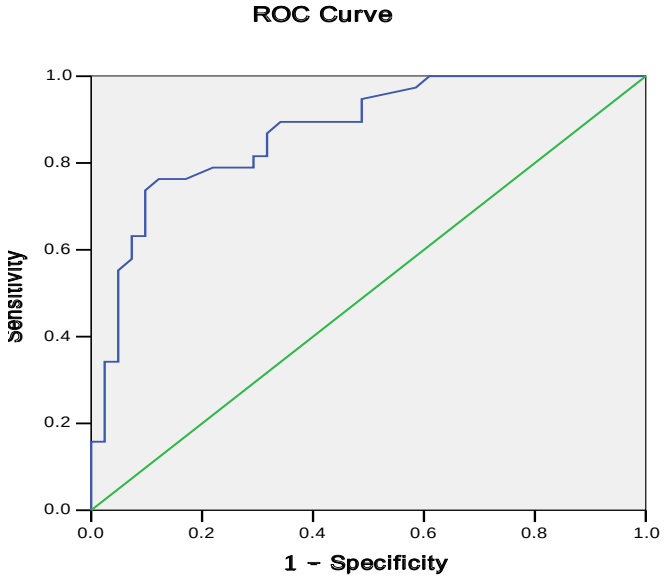


Figure -3. Association among first day of IL-1β and short outcome of asphyxia



An IL-1β concentration greater than 4 pg /dl had a sensitivity of 77%, a specificity of 83%, and positive predictive value of 71.4% and negative predictive value 78.4% in predicting the development of asphyxia

Figure - 4.Receiver operator curve (ROC) for serum IL-1β in prediction of asphyxiated group

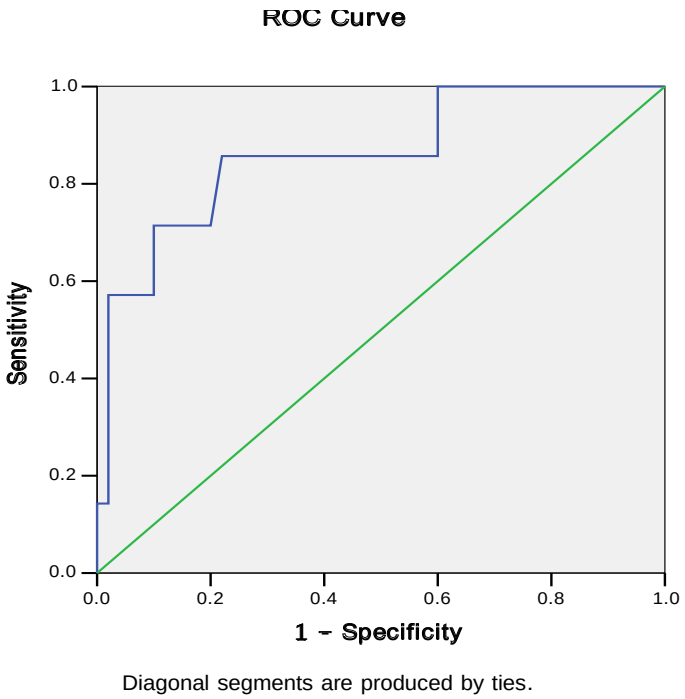


Figure - 5. Receiver operator curve (ROC) for serum IL-1β in prediction of adverse outcome.

a sensitivity of 77%, a specificity of 83%, positive predictive value of 71.4% and negative predictive value 78.4% in predicting the development of asphyxia (Figures 4, 5).

Discussion

In this study, we determined serum IL-1β concentrations on the first, second and third days of life; they were significantly increased after birth asphyxia, and these elevated concentrations were associated with poorer outcomes.

Association between asphyxia and IL-1β: Serum concentrations of IL-1β were elevated on the first day of life as compared with healthy control subjects. In animal studies, expression of both forms of IL-1 (IL-1β and IL-1α) are increased rapidly in response to experimental or clinical insults, such as head injury and cerebral ischemia. (6, 7) Elevated levels of certain cytokine (IL-1β, IL-8, and IL-6) responses have been reported in several clinical studies conducted on infants at term. (8-10) In our study, we prospectively measured serum IL-1β concentrations from asphyxiated neonates and age-matched healthy controls at three fixed time-points postpartum (the first, second and third days of life). We found that serum IL-1β levels were considerably higher in neonates with birth asphyxia ($p< 0.001$). This may be because pro-inflammatory cytokines, such as IL-1β are potential mediators of asphyxia. We found an association between short-term outcome asphyxia and IL-6; we found a considerable increase of serum IL-1β in asphyxiated infants; its concentration was predictive of short-term outcome. The role of cytokines in the pathogenesis of brain injury and their relation to neurological outcomes of asphyxiated neonates is not fully understood. Aly et al. have reported that the concentrations of IL-1β in cerebrospinal fluid after perinatal asphyxia are related to neurologic outcomes. IL-1β is known to have neurotrophic and neuroprotective effects. However , it is not clear whether IL-1β participates in the degeneration or repair of neurons after ischemic brain injury. (11) The mechanism of delayed injury is still unclear, but in the brains of infants dying after birth asphyxia, cells can be detected which show the hallmarks of apoptotic death. (12, 13) However, the authors did not define the cut-off value of serum IL-1β for predicting adverse outcomes. The results of the present study give additional support to their study, as well as defining the cut-off values of IL-1β in serum. In our study, serum IL-1β concentration greater than 6.7 pg/ ml had a sensitivity of 85.7% and a specificity of 78.0% in predicting adverse outcomes (Figure 5).

Conclusion

IL-1β may have important roles following injury to the CNS and serum concentrations appear to be a predictor of short-term neurological outcomes and severity of perinatal asphyxia. But more investigations are required to understand the role of this cytokine in cerebral injury caused by hypoxic insult.

Acknowledgment

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References

1. Costello AM, Manandhar DS. Perinatal asphyxia in less developed countries. Arch Dis Child Fetal Neonatal Ed 1994; 71: 1-3.
2. Ceccon M. Interleukins in hypoxic-ischemic encephalopathy. J Pediatr (Rio J) 2003; 79: 280-281.
3. Volpe J J. Neurology of the Newborn. 3rd ed. Philadelphia: W.B. Saunders; 2001. p. 217-394.
4. Oygur N, Sonmez O, Saka O, Yegin O. Predictive value of plasma and cerebrospinal fluid tumour necrosis factor-alpha and interleukin-1 beta concentrations on outcome of full term infants with hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal 1998; 79: 190-193.
5. Silverstein FS, Barks JD, Hagan P, Liu XH, Ivacko J, Szaflarski J. Cytokines and perinatal brain injury. Neurochem Int 1997; 30: 375-83.
6. Touzani O, Boutin H, LeFeuvre R, Parker L, Miller A, Luheshi G, et al. Interleukin-1 influences ischemic brain damage in the mouse independently of the interleukin-1 type I receptor. J Neurosci 2002; 22: 38-43.
7. Rothwell NJ. Annual review prize lecture cytokines - killers in the brain? J Physiol 1999; 514: 3-17.
8. Shalak LF, Laptook AR, Jafri HS, Ramilo O, Perlman JM. Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. Pediatrics 2002; 110: 673-680.
9. 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. Pediatr Neurol 2004; 31: 326-332.
10. Xanthou M, Fotopoulos S, Mouchtouri A, Lipsou N, Zika I, Sarafidou J. Inflammatory mediators in perinatal asphyxia and infection. Acta Paediatr Suppl 2002; 91: 92-97.
11. 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: 178-182.
12. Oygur N, Sonmez O, Saka O, Yegin O. Predictive value of plasma and cerebrospinal fluid tumour necrosis factor-alpha and interleukin-1 beta concentrations on outcome of full term infants with hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed 1998; 79: 190-193.
13. Mehmet H, Edwards AD. Hypoxia, ischaemia, and apoptosis. Arch Dis Child Fetal Neonatal Ed 1996; 75: 73-75.