Original Article

Combination of Serum Interleukin-1β and 6 Levels in the Diagnosis of Perinatal Asphyxia

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Abstract

Background: Perinatal asphyxia is an important cause of death, as well as permanent neurological and developmental complications. Diagnosing in time would lead to better prognosis and applying the most proper treatment. We sought to define the predictive values of serum concentrations of interleukin-1β (IL-1β) and interleukin-6 (IL-6) in newborns with perinatal asphyxia to see if there is a relation between the short-term neurological deficit and serum IL-1β and IL-6 concentrations.

Methods: This was a prospective (case-control) study conducted between March 2006 and April 2013, at the Neonatal Intensive Care Unit, Mashhad, Iran. Serum IL-1β and IL-6 levels were measured at birth in 38 consecutive uninfected neonates with perinatal asphyxia (blood pH < 7.2, low Apgar score, signs of fetal distress) and 47 randomly selected healthy newborns. The results were compared between the groups, using Chi-Square, *t*-tests, and Mann-Whitney tests, as well as receiver operator characteristics (ROC) curves and regression models.

Results: Serum IL-1β and IL-6 concentrations in the infants who developed perinatal asphyxia were significantly higher compared to values in the normal infants [16.88 *vs* 3.34 pg/mL for IL-1β, (P = 0.006), and 88.15 *vs* 6.74 pg/ mL for IL-6, (P < 0.001) respectively]. The sensitivity and specificity for the diagnosis of perinatal asphyxia using serum IL-6 were 80.5% and 81.6% respectively. The sensitivity and specificity using serum IL-1β were 71% and 89.1%, respectively.

Conclusion: Evaluating serum IL-6 and 1β simultaneously, could improve the sensitivity and specificity of early diagnosis of the perinatal asphyxia. The most appropriate indicator of perinatal asphyxia is combined measurement of interleukin 1β and interleukin 6.

Keywords: Asphyxia, diagnosis, infant, perinatal, serum

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Introduction

Perinatal asphyxia (PA) is defined as a shortage of oxygen occurring before, during or after birth, and has been linked to an increased risk for neurodevelopmental disabilities.[1,2] PA remains a common and serious problem, affecting about 1% of infants with approximately 1,000,000 neonatal deaths being attributable to PA every year globally. PA may have adverse impacts on all major body systems, in many cases leading to respiratory distress syndrome, disseminated intravascular coagulation, subcutaneous fat necrosis, myocardial ischemia, adrenal hemorrhage, metabolic disorders, or acute tubular necrosis.[3,4]

The mortality and morbidity rates among patients with moderate or severe hypoxic ischemic encephalopathy (HIE) are very high; 50% of patients with severe HIE die and almost all survivors develop neurodeficits.[5]

Several measures (ie. intrapartum electronic fetal monitoring, fetal or umbilical cord pH measurement, meconium-stained amniotic fluid, Apgar score, HIE, and major organ disorder) have been used either to predict or to define PA; However, they singly are insufficiently reliable and currently only a combination of parameters is clinically useful for the early detection of PA[6] and hence, other early biomarkers of PA need to be identified. Some researchers have investigated the use of specific biochemical markers like interleukins, heat shock proteins (HSPs)[7] prooxidant-antioxidant balance (PAB),[3] and hematologic markers (umbilical blood nucleated red blood cell (NRBC) count)[8] for the early detection of damage.

There is evidence supporting the involvement of inflammation in the pathogenesis of ischemic brain injury. The acute inflammation triggered by ischemia of the central nervous system (CNS) is characterized by polymorphonuclear cell recruitment, which needs the expression of unique adhesion molecules and chemotactic elements, and it would be continued by monocytes and microglial activation.[9,10] Lymphocytes, monocytes and macrophages combine and secret interleukins in relation to stimuli.[9] Experimental models suggest that several cytokines especially IL-6 are involved in ischemic brain damage. Fotopoulos, et al. have reported that NRBC absolute numbers, as well as serum IL-1β and IL-6 cytokine levels at 24 hours postnatally are significantly higher in neonates with PA/ infection compared to controls (P = 0.022, P = 0.036 and P = 0.037, respectively).[11]

There have been few studies that have investigated the association between serum IL-6 and IL-1β in HIE. Furthermore, there is insufficient evidence to validate the use of these cytokines in

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the prediction of clinical outcomes. This comparative study of the predictive values of serum IL-1β and IL-6 in neonates with PA was conducted to examine the potential diagnostic value as well as their association with the severity of perinatal asphyxia and short-term neurological outcomes.

Materials and Methods

Study population

This prospective case-control study was conducted at the Neonatal Intensive Care Unit, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, between March 2006 and April 2013. The evaluations were performed after obtaining parents' informed consents with ethical approval from the vice chancellor for research, Mashhad University of Medical Sciences.

Inclusion criteria

The inclusion criteria were the same as described in our previous work. PA was defined as presence of a minimum of two out of the five following clinical manifestations:

1. Signs of fetal distress (HR < 100, late decelerations, or heart rate invariability).
2. Meconium-stained amniotic fluid and hypoventilation, low muscular tone, or bradycardia.
3. An Apgar score ≤ 4 and ≤ 6 at 1 and 5 minutes after birth, respectively.
4. Need for resuscitation immediately after birth for more than one minute with oxygen and positive-pressure ventilation.
5. Acidosis (pH < 7.20 or a base deficit of > 12 mmol/L) during the first hour after birth.

Clinical assessment

Neonatal neurologic assessment including mental status (level of alertness), cranial nerve, and motor and sensory system functions were performed at days 1, 3 and 7. Examination of muscle tone and spontaneous movements were done as motor assessment. The active tone, posture and muscle resistance to passive movements were also evaluated. All neurologic examinations were performed by the same neonatologist. HIE was classified as mild (hyperexcitability, hypervigilance, or overactive reflexes with no seizures at least for the first 24 hours after birth); moderate (lethargy, low muscle tone, weak primitive reflexes, miosis, and seizures); and severe (apneustic breathing, flaccid paralysis, recurrent seizures, decelerated posture, and coma) according to Sarnat criteria.

In addition to HIE induced neurologic dysfunction, ventilator dependence or need for supplemental oxygen for > 24 h; congestive heart failure not associated with structural heart disease or shock; gut ischemia; elevated hepatic transaminases; prolonged PT or PTT; thrombocytopenia, acute tubular necrosis, and oliguria (urine < 1 mL/kg/hour) beyond 24 h were endpoints of systemic complications within the first weeks of life. The outcomes were classified as favorable and adverse. A favorable outcome was defined as normal neurologic and good general health by the end of the first month. An 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.

Laboratory measurement

After blood sample collection (1 – 2 mL) for cases (n = 38) and controls (n = 47) the serum was separated by centrifugation, and stored at -70°C prior to analysis. Serum IL-1β and IL-6 levels were measured using a highly sensitive and specific enzyme-linked immunosorbent assay kit (Bender Med Systems®, Austria). The minimum detectable concentration for IL-1β and IL-6 were 0.1 pg/mL, respectively. All samples were run in duplicates. Blood, cerebrospinal fluid cultures as well as serum creatinine, Na^+, K^+, Ca^{++} and IL-1β were determined at the time of the initial evaluations.

Statistical analysis

All statistical analyses were performed with Statistical Package for the Social Sciences 16.5 (SPSS Science, Apache Software Foundation, and Chicago, IL, USA). Values were expressed as mean ± SD. Student *t*-test, Kruskal-Wallis test and Mann-Whitney test were used as appropriate. Parametric and non-parametric correlations were assessed using Pearson and Spearman correlation coefficients, respectively. Sensitivity and specificity were calculated for IL-6 and IL-1β. Receiver-operating characteristic (ROC) curves were used for determination of thresholds for the asphyxic versus healthy neonate groups. Regression models were also applied for determination of the diagnostic markers. A $P < 0.05$ was considered statistically significant.

Results

Eighty-five subjects completed the study (n = 38 cases and n = 47 controls). No significant differences were found between the two groups regarding gender, gestational age and maternal age ($P > 0.05$, Table 1). Cases had significantly lower APGAR scores in the first five minutes postpartum, longer hospital stay, the likelihood of cesarean section, pregnancy or delivery complications compared to the control group ($P < 0.001$, Table 1).

Of the 38 infants with perinatal asphyxia, 5 infants had no HIE, 15 had HIE grade1, 11 had grade 2, and 7 had grade 3. The concentrations of the serum first-day IL-1β were considerably higher in the asphyxia group compared to the control group (16.88 *vs.* 3.34 pg/mL) ($P = 0.006$). Also the concentrations of the serum first day IL-1β were 3.34 pg/mL for controls, 4.45 pg/mL for asphyxiated neonates without HIE, 11.54 pg/mL for HIE grade 1, 14.63 pg/mL for HIE grade 2, and 46.58 pg/mL for HIE grade 3.

IL-6 serum concentrations were considerably higher in the asphyxic group ($P < 0.001$) compared to the controls [88.15 pg/mL *vs.* 6.74 pg/mL]. The concentrations of first day serum IL-6 were 6.74 pg/mL for controls, 48.96 pg/mL for asphyxic neonates without HIE, 58.97 pg/mL for grade 1 HIE, 131.66 pg/mL for grade 2 HIE, and 110.28 pg/mL for grade 3 HIE. A serum IL-6 > 11.91 pg/mL was associated with 80.5% sensitivity and 81.6% specificity in diagnosis of perinatal asphyxia. A serum IL-1β > 3.35 pg/dL was associated with 71% sensitivity and 89.1% specificity (see Figure 1).

According to data analysis by regression models, variables such as serum IL-6 ($P < 0.001$) and IL-1β ($P < 0.001$) were both significantly related to the subsequent diagnosis of perinatal asphyxia (Table 2). Asphyxic infants had the following clinical features: reduced reflexes (63%), loss of consciousness (45%), seizures (21%), respiratory distress (32%), apnea (11%), and cardiomegaly (24%).

Table 1. Clinical characteristics of the study population

	Control group (n = 47)	Case group (n = 38)	*P*-value
Gender			
Male	25 (53.2%)	16 (42.1%)	0.211
Female	22 (46.8%)	22 (57.9%)	
Gestational age (week)	39.43 ± 1.80	38.21 ± 2.23	0.087
Maternal age (y)	26.69 ± 5.24	26.32 ± 5.81	0.770
Birth weight (gr)	3032.27 ± 609.62	2444.69 ± 930.50	0.003
Mode of delivery (ND/CS)	33 (71.74%)/14 (28.26%)	13 (34.21%)/25 (65.79%)	0.001
First minute Apgar score	9 (1)	5 (3)	< 0.001
Fifth minute Apgar score	9 (0)	6 (2)	< 0.001
Duration hospital stay (d)	6 ± 4.58	7.05 ± 4.63	0.654

Values are expressed as means ± SD or number (%) or Median (interquartile)

Table 2. Laboratory characteristics of the study population

	Control group	Case group	*P*-value
White blood cell count	14682 ± 7104	16529 ± 6568	0.342
pH 1 hour post-partum	7.28 ± 0.03	7.14 ± 0.15	< 0.001
IL-1β	3.37 ± 4.53	17.46 ± 36.21	< 0.001
IL-6	6.74 ± 16.47	88.15 ± 84.86	< 0.001

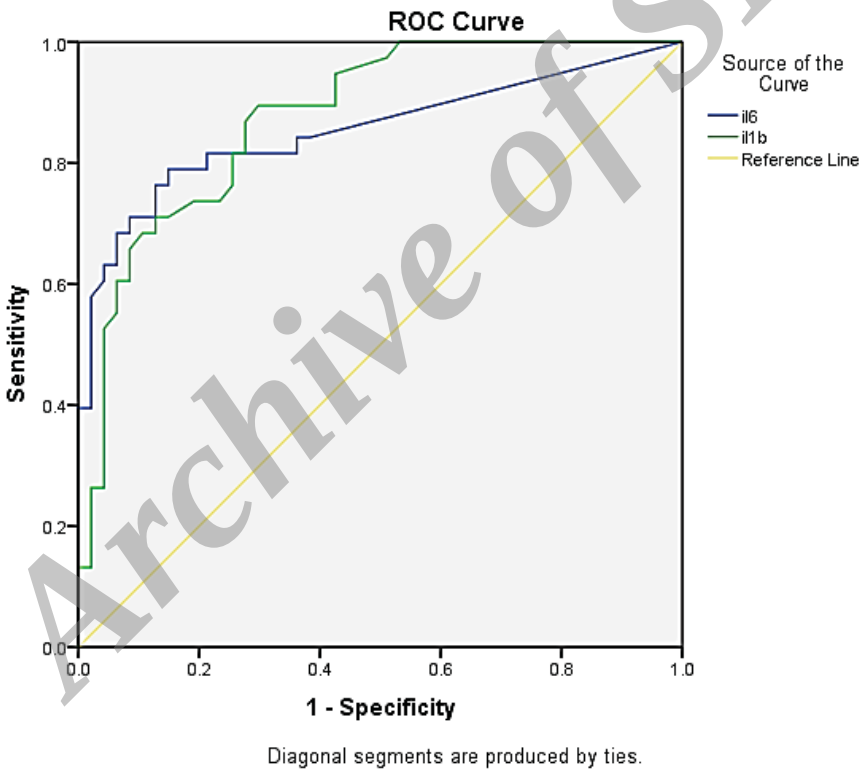

Figure 1. Receiver operating characteristics (ROC) graph to discriminate the sensitivity and specificity of serum IL-6 and IL-1β level for the diagnosis of perinatal asphyxia.

Among 38 infants with PA, 23 had favorable outcome (neurologic development was normal), and 15 had an adverse outcomes (eight died within the first month of life and seven had neurodevelopment sequelae). Serum IL-6 concentrations were significantly higher in neonates with adverse outcomes compared to those with favorable outcome (152.5 ± 79.91 pg/mL *vs.* 58.6 ± 64.8, $P = 0.001$). Serum IL-1β levels among infants with a favorable prognosis and those with unfavorable prognoses were 9.6 ± 8.2 and 29.8 ± 49.5, respectively, although the difference was not statistically significant ($P = 0.073$).

An IL-6 concentration > 28 pg/mL had a sensitivity of 93%, a specificity of 40% for predicting an adverse outcome. Serum IL-1β concentrations of > 6.5 pg/mL had a sensitivity of 92.8% and a specificity of 47% in predicting the adverse outcome.

Three possible models for predicting neonatal asphyxia are compared in Table 3. The use of serum interleukin-1β + interleukin-6 in combination appeared to provide the best model.

Table 3. Evaluation of the biochemical markers (IL-6 and IL-1β) for diagnosis of perinatal asphyxia, according to the regression model

Diagnostic methods	-2log likehood	Cox & Snell R Square	Nagelkerke R Square	Predicted Percentage Correct	Hosmer & Lemeshow Test (sig)
Interleukin-1β	86.159	0.303	0.406	78.8	0.086
Interleukin-6	75.480	0.386	0.516	81.2	0.065
Interleukin-1β with Interleukin-6	67.56	0.440	0.589	84.2	0.078

Discussion

In this study, we found that a combination of serum IL-1β and IL-6 levels was an effective way for assessing perinatal asphyxia. Serum IL-6 and IL-1β concentrations were significantly higher in neonates with PA compared to the healthy controls on the first post-natal day, and these elevated concentrations were associated with the severity of asphyxia and a poorer outcome. The elevated serum IL-6 and IL-1β levels could be indicative of the involvement of this cytokine as a potential mediator of asphyxia.

Previous studies have reported elevated levels of serum IL-1β, IL-8 and IL-6 in infants with asphyxia at term.[12–16]

Animal studies have shown a rapid increase in the expressions of IL-1β and IL-1α in response to experimental or clinical insults such as head injury and cerebral ischemia.[17,18] Elevated serum levels of certain cytokine (IL-1β, IL-8, and IL-6) have also been reported in several clinical studies conducted on infants at term.[12–14]

A significant association was observed between serum IL-6 concentrations and Sarnat's grading of the severity of encephalopathy among neonates with HIE. A recent study has reported high serum IL-6 levels in infants with HIE.[12] However, the authors did not define the cut-off values of serum IL-6 that were predictive of long-term adverse outcomes. Our results are also in line with the findings of Aly, et al.[19] who reported a significant correlation between serum IL-6 concentrations and Sarnat's grading of encephalopathy. IL-6 appears as a critical product among inflammatory cytokines in pathogenesis of perinatal asphyxia.[20]

Aly, et al. have also claimed that elevated concentrations of IL-1β in cerebrospinal fluid lead to neurologic outcomes after perinatal asphyxia. It can be inferred that IL-1β has neurotrophic and neuroprotective results. However, it is not clear whether IL-1β participates in the degeneration or repair of neurons after ischemic brain injury.[19]

Increased serum IL-6 levels during the first 24 hours after hypoxic ischemic insult would lead to an augmentation in its role in pathogenesis of brain injury.

It is also possible that IL-6 might be released as a protective response after hypoxic ischemic brain injury and is a cytokine with both pro- and anti-inflammatory potentials.[12,21] Further studies will help to more investigation of putative bimodal action of IL-6 functions in the pathogenesis of HIE.[12] However, it is not clear whether IL-6 have a degenerative or reconstructive effect on neurons after ischemic brain injury.[19] In a previous study by Tekgul, et al.[13] serum IL-6 yielded a positive 86% predictive value with 100% specificity in prediction of moderate to severe HIE. Another study has reported elevated serum IL-6 concentrations among perinatal asphyxic neonates are associated with poor outcomes or death.[16] Nelson and Grether[22] used dried neonatal blood samples from children with spastic cerebral palsy and matched controls in a retrospective study and reported elevations in serum IL-6, IL-11, and IL-13. These cytokines showed a sensitivity and specificity of > 88% in diagnosis of cerebral palsy.[23]

In conclusion, serum IL-6 and IL-1β concentrations are significantly higher in birth asphyxia and the magnitude of the elevations are associated with the severity of encephalopathy. A combined measurement of interleukin-1β and interleukin-6 is the most appropriate indicator of perinatal asphyxia.

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References

1. van Handel M, Swaab H, de Vries LS, Jongmans MJ. Long-term cognitive and behavioral consequences of neonatal encephalopathy following perinatal asphyxia: A review. *European Journal of Pediatrics*. 2007; 166(7): 645 – 654.
2. Boskabadi H, Tavakol Afshari J, Maamouri G, Ghayour-Mobarhan M, Ferns. Association between serum interleukin-6 levels and severity of perinatal asphyxia. *Asian Biomedicine*. 2010; 4(1): 79 – 85.
3. Boskabadi H, Navaee Boroujeni A, Mostafavi-Toroghi H, Hosseini G, Ghayour-Mobarhan M, Hamidi Alamdari D, et al. Prooxidant-antioxidant balance in perinatal asphyxia. *Indian Journal of Pediatrics*. 2014; 81(3): 248 – 253.
4. Boskabadi H, Ashrafzadeh F, Doosti H, Zakerihamidi M. Assessment of risk factors and prognosis in asphyxiated infants. *Iran J Pediatr.* 2015; 25(4): 1 – 6.
5. Dixon G, Badawi N, Kurinczuk JJ, Keogh JM, Silburn SR, Zubrick SR, et al. Early developmental outcomes after newborn encephalopathy. *Pediatrics*. 2002; 109(1): 26.
6. Boskabadi H, Maamouri G, Rezagholizade Omran F, Mafinejad S, Tara F, Rayman MP, et al. Effect of prenatal selenium supplementation on cord blood selenium and lipid profile. *Pediatrics and Neonatology*. 2012; 53(6): 334 – 339.
7. Boskabadi H, Omidian M, Tavallaie S, Mohammadi M, Parizadeh M, Ghayour-Mobarhan M, et al. Serum Hsp70 antigen: Early diagnosis marker in perinatal asphyxia. *IJP*. 2015; 25(2): e381.
8. Boskabadi H, Maamouri G, Sadeghian MH, Ghayour-Mobarhan M, Heidarzade M, Shakeri MT, et al. Early diagnosis of perinatal asphyxia by nucleated red blood cell count: A case-control study. *Arch Iran Med*. 2010; 13(4): 275 – 281.
9. Ceccon MEJR. Interleukins in hypoxic-ischemic encephalopathy. *Journal de Pediatria*. 2003; 79(4): 280 – 281.
10. Silveira RC, Procianoy RS. Interleukin-6 and tumor necrosis factor-[alpha] levels in plasma and cerebrospinal fluid of term newborn infants with hypoxic-ischemic encephalopathy. *The Journal of Pediatrics*. 2003; 143(5): 625 – 629.
11. Fotopoulos S, Pavlou K, Skouteli H, Papassotiriou I, Lipsou N, Xanthou M. Early markers of brain damage in premature low-birth-weight neonates who suffered from perinatal asphyxia and/or infection. *Biology of the Neonate*. 2001; 79(3-4): 213 – 218.
12. Shalak LF, Laptook AR, Jafri HS, Ramilo O, Perlman JM. Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. *Pediatrics*. 2002; 110(4): 673.
13. 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. *Pediatric Neurology*. 2004; 31(5): 326 – 332.
14. Xanthou M, Fotopoulos S, Mouchtouri A, Lipsou N, Zika I, Sarafidou J. Inflammatory mediators in perinatal asphyxia and infection. *Acta Paediatrica*. 2002; 91: 92 – 97.
15. 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. *European Journal of Clinical Investigation*. 2003; 33(4): 352 – 358.
16. Okazaki K, Nishida A, Kato M, Kozawa K, Uga N, Kimura H. Elevation of cytokine concentrations in asphyxiated neonates. *Neonatology*. 2005; 89(3): 183 – 189.
17. Rothwell NJ. Annual review prize lecture cytokines-killers in the brain? *The Journal of Physiology*. 1999; 514: 3.
18. 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. *The Journal of Neuroscience*. 2002; 22(1): 38.
19. Aly H, Khashaba MT, El-Ayouty M, El-Sayed O, Hasanein BM. IL-1 [beta], IL-6 and TNF-[alpha] and outcomes of neonatal hypoxic ischemic encephalopathy. *Brain and Development*. 2006; 28(3): 178 – 182.
20. 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. *The Journal of Immunology*. 1990; 144(1): 129.
21. Kilpinen S, Hulkkonen J, Wang XY, Hurme M. The promoter polymorphism of the interleukin-6 gene regulates interleukin-6 production in neonates but not in adults. *European Cytokine Network*. 2001; 12(1): 62 – 68.
22. Nelson KB, Grether JK. Causes of cerebral palsy. *Current Opinion in Pediatrics*. 1999; 11(6): 487.
23. Foster-Barber A, Dickens B, Ferriero DM. Human perinatal asphyxia: correlation of neonatal cytokines with MRI and outcome. *Developmental Neuroscience*. 2000; 23(3): 213 – 218.