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Original article

Association between serum interleukin-6 levels and severity of perinatal asphyxia

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Background: Perinatal asphyxia is a major cause of neurologic morbidity and mortality in infants.
Objective: Determine the serum level of interleukin-6 (IL-6) in neonates with perinatal asphyxia and its relation to the severity of hypoxic-ischemic encephalopathy and short term neurological outcome.
Methods: Serum IL-6 levels were measured at birth, and at 24 and 48 hour post-partum in 37 consecutive uninfected neonates with peri-natal asphyxia and 45 randomly selected healthy newborns.
Results: Serum IL-6 concentrations in the infants who developed hypoxic-ischemic encephalopathy was 43 folds higher compared to values in the normal infants ($p < 0.001$) and 1.9 folds higher as compared to infants with asphyxia who did not subsequently develop hypoxic-ischemic encephalopathy ($p < 0.001$). Serum IL-6 concentrations were also related to the degree of hypoxic-ischemic encephalopathy and neurological-developmental outcomes at the time of discharge.
Conclusion: Serum levels of IL-6 increased in neonates with asphyxia, and this was most pronounced in neonates with adverse outcomes.

Keywords: Hypoxic-ischemic encephalopathy, interleukin-6, perinatal asphyxia, newborn

Perinatal asphyxia is a common cause of infant morbidity and mortality in neonatal period and longer-term neurologic disabilities [1]. It is estimated that 2-4/1000 full-term neonates suffer asphyxia at or shortly before birth. Approximately 15% to 33% of infants developing hypoxic-ischemic encephalopathy (HIE), die during the neonatal period, and 25% of the survivors will exhibit permanent neuropsychologic deficits [2, 3]. 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 [4]. 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 polymorphonuclear cell recruitment, requiring the expression of specific adhesion molecules and chemotactic factors, and is followed by monocytes and microglial activation [2, 5]. Interleukins are synthesized and secreted in response to stimuli by lymphocytes, monocytes, and macrophages [2]. Experimental models suggest the involvement of cytokines, especially IL-6, in ischemic brain damage. Although cytokines appear to modulate the apoptosis

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of CNS cells, promoting differentiation, proliferation, and subsequent leukocyte infiltration, the exact role of pro-inflammatory cytokines such as IL-6 as potential mediators during the progression of brain injury is unclear [6].

To our knowledge, there are only few previous studies [7-9] that have evaluated the association between hypoxia-ischemia and the release of cytokines. Furthermore, there is insufficient evidence to validate the utility of these cytokines in the prediction of clinical outcomes [10, 11]. In the present study, we investigated the association between serum IL-6 concentration with clinical outcomes and severity of perinatal asphyxia.

Methods

Study population

This was a prospective case-control study conducted between June 2007 and July 2008, in Ghaem Hospital, Mashhad, Iran. The study was undertaken with the approval of the Ethics Committee of the Mashhad University of Sciences, and parental informed consent was obtained for every neonate before inclusion in the study. Perinatal asphyxia was defined as the presence of at least two of the following conditions:

1) Signs of fetal distress (heart rate of 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 (determined by evaluating the following parameters of the newborn: heart rate, respiratory effort, muscle tone, response to catheter in nostril and color) of four or less at one minute or six or less at five minutes.

4) A need for resuscitation for more than one minute with positive-pressure ventilation and oxygen immediately after birth.

5) Blood pH value of less than 7.20 or a base deficit of at least 12 within the first hour after birth.

Fifty cases were originally eligible for inclusion in this study, but 13 were excluded because of insufficient blood sample (n=3), congenital or perinatal infection (n=2), histologic or clinical chorioamnionitis (maternal temperature during labor greater than 38°C and foul-smelling amniotic fluid) (n=5), maternal drug addiction (n=1) and congenital malformation (n=2). Only one infant in the case group did not complete one month of the follow-up. Fifty healthy neonates (defined as those neonates who were free of a postnatal clinical event during the first week of life) were also recruited as the control group. Among these fifty control neonates, five were excluded due to the incomplete data.

Clinical assessment

Neurological functions of the neonates were assessed at birth, and on the second and seven days of life. These 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. Newborn neurological examinations were performed by a single Neonatologist experienced in neurological evaluation without knowledge of the cytokine concentration.

The infants with perinatal asphyxia were subsequently classified dependent on whether they developed HIE within the first seven days after birth. According to the criteria by Sarnat [12], HIE was classified as mild (grade 1) if hyperexcitability or hyper-alerty or hyper-reflexia persisted without seizures for at least 24 hours after birth; as moderate (grade 2) if the infant was lethargic, had hypotonia, weak primitive reflexes, pupil miosis and seizures; and as severe (grade 3) if the infant had apnoea, flaccid weakness, frequent seizures, decelerated posture, or coma.

In addition to the neurologic dysfunction arising from HIE, endpoints of systemic complications within the first weeks of life included: pulmonary ventilator dependence or the need for supplemental oxygen for >24 hours; congestive heart failure not associated with structural heart disease, or shock, gut ischemia, elevated transaminases, prolonged prothrombin time or partial thromboplastin time, thrombocytopenia, acute tubular necrosis, or oliguria (<1 mL/kg/hour urine flow rates) beyond 24 hours. The outcome was classified as favorable or adverse. A favorable outcome was defined as normal if there was normal neurological development 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, unreliable sucking, seizures resistant to Phenobarbital, and sensory neural hearing loss.

Laboratory measurements

Blood samples (1-2 mL) from case (n=37) and control (n=45) neonates were collected on the first, second and third days of life. Serum was separated by centrifugation, and then stored in aliquots at -70°C until analysis. IL-6 levels were measured using a highly sensitive and specific enzyme-linked immunosorbent assay kit (Bender MedSystems®, GmbH, Vienna, Austria). The minimum detectable concentration for IL-6 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-6 were determined at the time of the initial evaluations in the neonates with asphyxia.

Statistical analysis

Descriptive statistics and analytical tests were performed using SPSS software. Data were presented as mean±SD or number (%). Establishment of a cut-off value between low and high levels of IL-6 was performed using a receiver operating characteristic (ROC) curve. A p-value of <0.05 was considered statistically significant.

Results

Among 100 neonates who were initially recruited into the study, 82 infants (37 cases and 45 controls) completed the study. There was no statistically significant difference between the two groups regarding weight, gender, gestational age, maternal age and maternal parity (p <0.05, **Table 1**). In comparison with the controls, the cases had a significantly lower APGAR score in the first minute and first five minutes post partum, longer hospital stay, likelihood of Cesarean section, pregnancy or delivery complications, and respiratory problem (p <0.001, **Table 1**).

Out of the 37 infants with perinatal asphyxia, three infants did not develop HIE, 15 developed HIE grade1, 11 grade 2, and eight grade 3. All infants in the asphyxia group had negative body fluid cultures, and received antibiotic treatment for five days or less.

The result of brain Computerized Tomography scan were normal in the three infants with no HIE as well as infants with grade 1 HIE. However, of the 19 infants with grade 2 or 3 HIE, eight were found to have diffused brain oedema, seven had homogenous hyperechogenicity, and three had subarachnoid hemorrhage.

Table 1. Clinical and biochemical characteristics of the study population.

Group	Asphyxia	Control	*P*-Value
Number	37	45	
Birth weight (g)	2892 ± 896	3036 ± 377	> 0.05
Gestational age (week)	37.9 ± 1.5	39.1 ± 1.6	> 0.05
One-minute Apgar	4.4 ± 1.5	8.56 ± 0.67	< 0.001
Five-minute Apgar	6.1 ± 1.5	9 ± 0.5	< 0.001
Mode of dlivery (ND/CS)	12/21	27/14	> 0.05
Duration hospital stay	7.93 ± 5	0.4 ± 1.4	< 0.001
Maternal age	26 ± 5.6	27 ± 5.1	> 0.05
Sex (male/female)	20/13	21/20	> 0.05
Pregnancy complication[a]	15 (65.2%)	8 (34.8%)	< 0.05
Delivery complication[b]	19 (57.6%)	1 (2.4%)	< 0.001
Respiratory problem[c]	17 (51.6%)	1 (2.4%)	< 0.001
Cardiac problem[d]	8 (25.8%)	0	< 0.001
abdomen problem[e]	4 (12.5%)	0	< 0.05
CPR in delivery room	9 (36.5%)	0	< 0.05
PH	7.12 ± 0.16	7.30 ± 0.14	< 0.001
Base exess	-9.71 ± 10.61	-4 ± 2.86	< 0.001

Values are expressed as means±SD or number (%). [a]eclampsia, pre-eclampsia, polyhydroaminus, epilepsy; [b]sufrance, placental apruption, placenta previa; [c]tachypnoea, Apnoea, granting; [d]cardiac murmur, bradycardia, cardiomegaly; [e]feeding intolerance, abdominal distention.

The concentrations of serum IL-6 in the first day were considerably higher in the asphyxia group compared with the control group ($p < 0.001$). Likewise, serum IL-6 concentrations in the first day was 119.5±81.1 pg/mL in the infants who subsequently developed HIE, being about 44 fold higher than that of normal infants (2.7±4.6 pg/mL, $p < 0.001$). Among the infants in whom HIE developed, first day serum IL-6 levels were 77.6±63.0 pg/mL in those with stage1,149.4±79.3 pg/mL with stage 2 and 170.8±78.3 pg/mL with stage 3. There was also a significant association between the serum level of the first day IL-6 and severity of HIE (**Fig. 1**).

The time course of IL-6 response during the immediately postnatal period in asphyxiated infants is previously unpublished. We found that in the whole group of asphyxiated neonates, as well as within the subgroup with or without HIE, median IL-6 was significantly lower at 48 hours of life than at birth, with a significant decline from 36-72 hours of life ($p < 0.001$). Seventeen of the neonates without asphyxia had adverse outcome. Among the 34 infants who had HIE, 17 had favorable outcome (neurologic development was normal), and 17 had adverse outcome (nine died within the first month of life, and eight had neurodevelopment sequelae). Median serum IL-6 concentrations were significantly higher in neonates with adverse outcome than in those with favorable outcome (170.18 ± 71.1 pg /mL *vs* 23.8 ± 45.4 pg/mL, $p < 0.001$).

An IL-6 concentration greater than 12.5 pg/mL had a sensitivity of 94.5%, a specificity of 97.7%, positive predictive value of 97.0%, and negative predictive value of 95.0% in predicting the development of asphyxia (**Fig. 2**). Serum IL-6 concentrations of greater than 41 pg/mL had a sensitivity of 93.7% and a specificity of 81.5% in predicting the adverse outcome. A serum IL-6 concentration >41 pg/mL and moderate or severe hypoxic ischemic encephalopathy had a sensitivity of 88.2%, a specificity of 96.9%, positive predictive value of 88.2%, and negative predictive value 96.9% in predicting the adverse outcome.

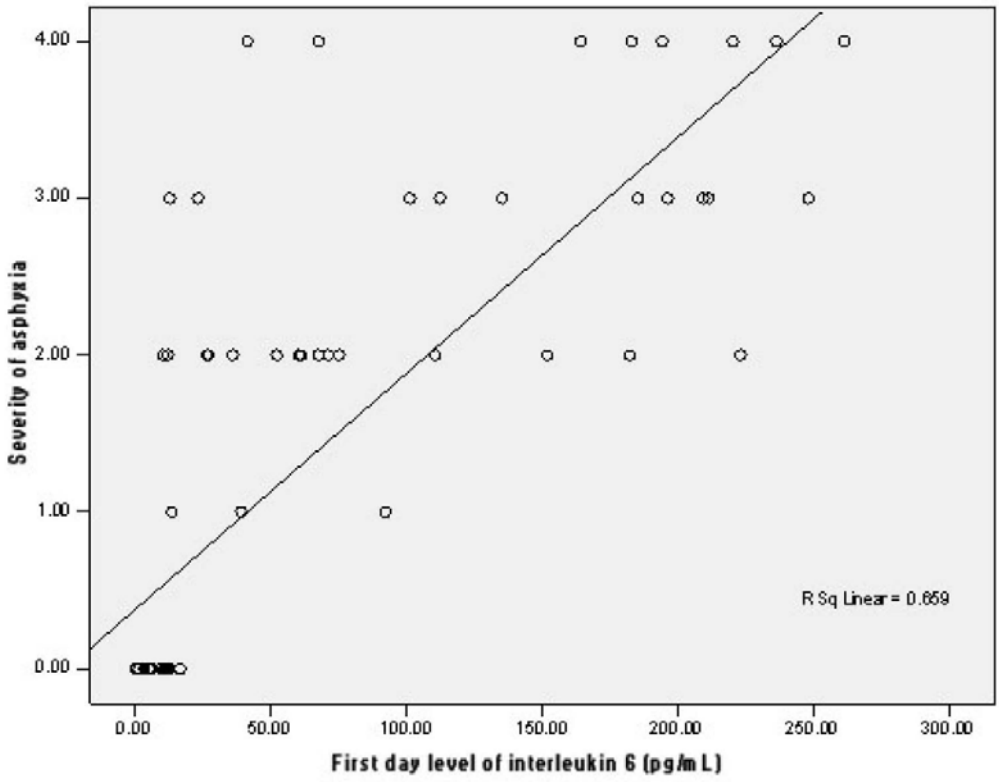

Fig. 1 Association between the first day level of IL-6 and severity of asphyxia. R Sq Linear = Linear Regression R-Squared function to determine the extent of a linear relationship between the two variables.

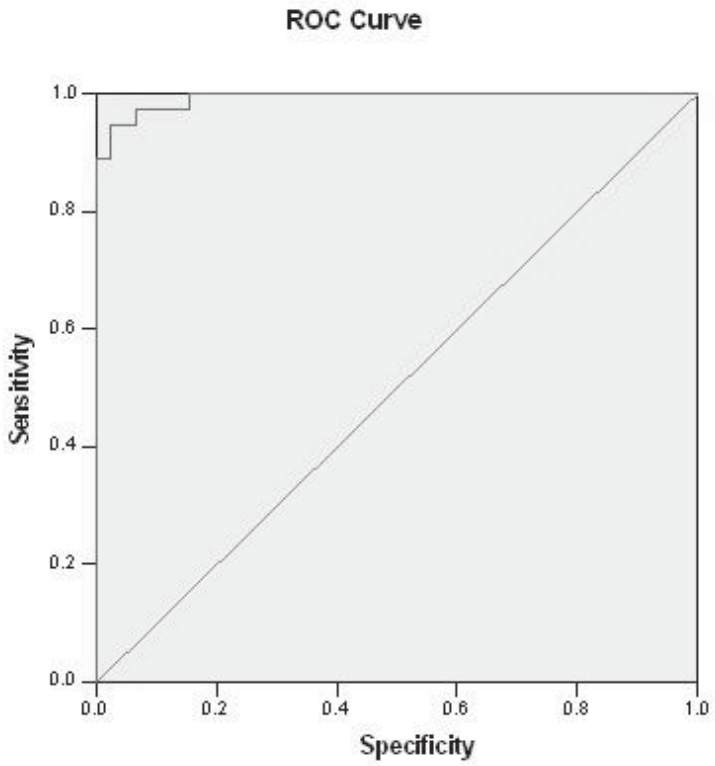

Fig. 2 Receiver operator curve (ROC) for serum IL-6 in prediction of asphyxia.

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 [13-15]. Similar to our findings, the concentrations of serum IL-6 have been reported to be higher in asphyxiated neonates than those of normal neonates [1, 15, 16]. However, we have studied a larger population and prospectively measured serum IL-6 concentrations in asphyxiated neonates and age-matched healthy controls at three fixed time points postpartum i.e., in the first, second and third day of life.

Concerning the neonates who were diagnosed with HIE, a significant association was observed between serum IL-6 concentrations and Sarnat's grading of the severity of encephalopathy. A recent study has reported high IL-6 levels in plasma of infants with HIE [13]. However, the authors did not define the cut-off values of serum IL-6 that were predictive of adverse outcome in the longer-term. The present results give additional support to their study, as well as defining the cut-off values of serum IL-6. Our results are also concordant with the finding by Aly et al. [17] who have reported that serum IL-6 concentrations were significantly correlated to the Sarnat's grading of encephalopathy. Moreover, we found that serum IL-6 concentrations were elevated in neonates who subsequently died, compared to those who survived. Serum IL-6 concentrations were also related to neurological outcomes at the end of the first month. These findings are almost in line with previous studies in which the concentrations of IL-6 in cerebrospinal fluid after perinatal asphyxia were related to early and late neurological manifestations [14, 18]. Besides, increased IL-6 levels have been reported in the serum and CSF of stroke patients [19, 20].

In experimental studies, rats with HIE were found to have peak serum IL-6 levels approximately six hours after the induction of HIE, with concentrations returning to basal levels after 20 hours [21]. To our knowledge, the time course of the cytokine response

after birth asphyxia has not been previously reported in human newborns. Our findings showed that serum IL-6 was significantly elevated by approximately 12 hours after birth.

Among the inflammatory cytokines, IL-6 appears as a critical product in the pathogenesis of hypoxic-ischemic brain injury [22]. The rise in serum IL-6 response within the first 24 hours after hypoxic-ischemic insult provides additional support for the possible role of this cytokine in the 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 involved in the repair mechanisms in the subacute stage of HIE [22-24]. Since IL-6 is a pleiotropic cytokine with both proinflammatory and anti-inflammatory potential [14, 25], Further studies are required to clarify this putative bimodal action of IL-6 functions in the pathogenesis of HIE [14].

In addition to the considerable increase of serum IL-6 in asphyxiated infants, we observed that these concentrations were predictive of short-term outcome. Previously, serum IL-6 concentrations were also found to be highly predictive of the subsequent degree of HIE, and adverse outcomes in the longer run in term infants with perinatal asphyxia [19, 26]. IL-6 is known to have neurotrophic and neuroprotective effects. Therefore, it is not clear whether IL-6 participates in the degeneration or repair of neurons after ischemic brain injury [17]. In a previous study by Tekgul et al. [14], serum IL-6 yielded a positive predictive value of 86% and 100% specificity for predicting moderate to severe HIE. In another investigation, serum IL-6 concentrations were higher in asphyxiated neonates with either a poor outcome or death [16]. In a retrospective study, Nelson and Grether [27] used dried neonatal blood samples from children with spastic cerebral palsy, most born at term, and gestationally age-matched controls and reported an elevation of IL-6, IL-11, and IL-13 in blood samples yielded a sensitivity and specificity >88% for the diagnosis of cerebral palsy. In addition, elevated serum levels of IL-6 were found in infants with changes characteristic of encephalopathy on magnetic resonance imaging (MRI) and who evolve to cerebral palsy [28]. In our study, the addition of serum IL-6 (>41 pg/mL) to the presence of moderate and severe hypoxic-ischemic encephalopathy allowed an improvement in the specificity and negative predictive value for adverse outcome.

In conclusion, we indicated 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

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. Ceccon ME. Interleukins in hypoxic-ischemic encephalopathy. J Pediatr (Rio J). 2003; 79:280-1.
3. Volpe JJ. Neurology of the Newborn. 3rd ed. Philadelphia:WB Saunders; 2001. p. 217-394.
4. De Costello LAM, Manandhar DS. Perinatal asphyxia in less developed countries. Arch Dis Child Fetal Neonatal Ed .1994; 71:F1-3.
5. 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. J Pediatr. 2003; 143: 625-9.
6. Dihne M, Block F. Focal ischemia induces transient expression of IL-6 in the substantia nigra pars reticulata. Brain Res. 2001; 889:165-73.
7. 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.
8. Thornberg E, Thiringer K, Hagberg H, Kjellmer I. Neuron specific enolase in asphyxiated newborns: association with encephalopathy and cerebral function monitor trace. Arch Dis Child Fetal Neonatal Ed. 1995; 72:F39-42.

9. 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.
10. Nagdyman N, Grimmer I, Scholz T, Muller C, Obladen M. Predictive value of brain-specific proteins in serum for neurodevelopmental outcome after birth asphyxia. Pediatr Res. 2003; 54:270-5.
11. Yoon BH, Jun JK, Romero R, Park KH, Gomez R, Choi JH, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1β, and tumor necrosis factor-α), neonatal brain white matter lesions, and cerebral palsy. Am J Obstet Gynecol. 1997; 177:19-26.
12. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol. 1976; 33: 696-705.
13. 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.
14. 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; 31:326-32.
15. Xanthou M, Fotopoulos S, Mouchtouri A, Lipsou N, Zika I, Sarafidou J. Inflammatory mediators in perinatal asphyxia and infection. Acta Paediatr Suppl. 2002; 91:92-7.
16. Okazaki K, Nishida A, Kato M, Kozawa K, Uga N, Kimura H. Elevation of cytokine concentrations in asphyxiated neonates. Biol Neonate. 2006; 89:183-9.
17. Aly H, Khashaba MT, El-Ayouty M, El-Sayed M, Hasanein BM. IL-1β, IL-6 and TNF-α and outcomes of neonatal hypoxic ischemic encephalopathy. Brain Development. 2006; 28:178-82.
18. Martin-Ancel A, Garcia-Alix A, Pascual-Salcedo D, Cabanas F, Valcarce M, Quero J. Interleukin-6 in the cerebrospinal fluid after perinatal asphyxia is related to early and late neurological manifestations. Pediatrics. 1997; 100:789-94.
19. Savman K, Blennow M, Gustafson K, Tarkowski E, Hagberg H. Cytokine response in cerebrospinal fluid after birth asphyxia. Pediatr Res. 1998; 43:746-51.
20. Tarkowski E, Rosengren L, Blomstrand C, Wikkelso C, Jensen C, Ekholm S, et al. Early intrathecal production of interleukin-6 predicts the size of brain lesion in stroke. Stroke. 1995; 26:1393-8.
21. Hagberg H, Gilland E, Bona E, Hanson LA, Hahin-Zoric M, Blennow M, et al. Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxia-ischemia in neonatal rats. Pediatr Res. 1996; 40:603-9.
22. 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. 1990; 144:129-35.
23. Maeda Y, Matsumoto M, Hori O, Kuwabara K, Ogawa S, Yan SD, et al. Hypoxia/reoxygenation-mediated induction of astrocyte interleukin-6: a paracrine mechanism potentially enhancing neuron survival. J Exp Med.1994; 180:2297-308.
24. Fassbender KRS, Kammer T, Daffertshofer M, Wirth S, Dollman M, Hennerici M. Proinflammatory cytokines in serum of patients with acute cerebral ischemia: kinetics of secretion and relation to the extent of brain damage and outcome of disease. J Neurol Sci. 1994; 122:135-9.
25. 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. Eur Cytokine Netw. 2001; 12:62-8.
26. Savman K, Blennow M, Hagberg H, Tarkowski E, Thoresen M, Whitelaw A. Cytokine response in cerebrospinal fluid from preterm infants with posthaemorrhagic ventricular dilatation. Acta Paediatr. 2002; 91:1357-63.
27. Nelson KB, Grether JK. Causes of cerebral palsy. Curr Opin Pediatr. 1999; 11:487-91.
28. Foster-Barber A, Dickens B, Ferriero DM. Human perinatal asphyxia: correlation of neonatal cytokines with MRI and outcome. Dev Neurosci. 2001; 23:213-8.