Leptin: Does It Have a Role in Neonatal Sepsis?

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Abstract: The principal aim of this study is to evaluate serum leptin role in neonatal sepsis and whether circulating leptin was related to and interleukin-6 (IL-6) and interleukin-1 (IL-1β) release in septic newborn infants, as well as to analyze the interaction between their levels, before and after antimicrobial therapy in neonates with bacterial septicemia. Serum leptin was measured in 14 neonates with sepsis as soon as sepsis was diagnosed and before treatment (group I) and after recovery (Group II) and in 14 healthy control infants. There was a highly significant increase in serum leptin levels between septic and control neonates (p<0.001); Furthermore, there was a very highly significantly decrease of the levels of serum leptin, IL-6 and IL-1β in neonates after recovery than before treatment (p<0.001). A very good relationship between leptin and IL-6; as well as IL-1β before and after therapy was identified. These findings suggest that a role of leptin in acute neonatal sepsis appears to be likely.

Key words: neonatal sepsis, serum leptin, interleukins, IL-6, IL-1β

INTRODUCTION

Bacterial sepsis is one of the major causes of neonatal morbidity and mortality. The incidence ranges from 1–10 per 1000 live births[1].

Diagnosis of neonatal sepsis may be difficult because clinical presentations are often nonspecific, bacterial cultures are time-consuming and other laboratory tests lack sensitivity and specificity[2].

It is vital to identify infected neonates as early as possible, but unreliable clinical signs and the absence of good diagnostic tests hinder an accurate early diagnosis[1]. Thus, sick neonates are frequently treated with broad-spectrum antibiotics, but true infection is only verified in a minority of cases[3,1]. Previously, various white blood cell counts and the acute phase reactant C-reactive protein (CRP) have been used to diagnose neonatal sepsis. The ratio between immature and total neutrophil cell count (I/T-ratio) is a sensitive, but not very specific, diagnostic method with substantial inter-observer variability[4]. CRP is specific, but less sensitive in the early stages of neonatal sepsis[5].

The inflammatory response is mediated by cytokines that are used as neonatal infection markers, especially interleukin-6 (IL-6). IL-6 is an inducer of hepatic protein synthesis, promotes production and liberation of C-reactive protein, and can be detected early when there is bacterial blood stream invasion. It acts as a signal for T-cell activation, promotes antibody secretion by B cells and differentiation of cytotoxic T cells, and stimulates liberation of other cytokines, particularly TNF- and IL-1[4,6,7,8].

Leptin, the 16-kDa protein product of the ob gene, is a pleotropic hormone that is produced primarily by adipocytes. In general, circulating leptin levels are linearly correlated with total body fat mass[9,10]. However, leptin levels drop rapidly during fasting and increase in response to infection and inflammatory stimuli[11,12]. In addition to regulating appetite and energy expenditure, leptin is an important immunoregulatory hormone since it enhances a number of immune responses, including macrophage effector functions[13,14] cytokine synthesis, and T helper (Th) cell polarization to a Th1 phenotype[15].

The principal aim of this study is to evaluate serum leptin role in neonatal sepsis and whether circulating leptin was related to and interleukin-6 (IL-6) and interleukin-1 (IL-1β) release in septic newborn infants, as well as to analyze the interaction between their levels, before and after antimicrobial therapy in neonates with bacterial septicemia.

MATERIALS AND METHODS

Methods: This case-control follow up study was conducted on fourteen full-term neonates with blood-culture positivity and clinical sepsis (Group I). They were hospitalized for clinical suspicion of neonatal sepsis in neonatal intensive care unit, Pediatric department, Zagazig University, Egypt. Those cases were followed up after recovery and enrolled in the

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study (group II). Another fourteen healthy neonates, of matched age and sex, followed at the neonatal units and outpatient clinics (group III) were chosen for sake of comparison. This prospective study was approved by the Medical ethical committee of the National Research Center. Parental consents were obtained.

Infants with malformations, chromosomal anomalies, erythroblastosis, diabetic or preeclamptic mothers, perinatal asphyxia (pH <7.20), or intrauterine growth retardation were excluded. Preterm newborns were also excluded from the study.

Complete obstetric history and physical examinations were obtained on admission. The demographic and clinical characteristics of the septic and healthy neonates were recorded. Signs and symptoms of sepsis were divided into six categories (adopted from Dollner *et al.,* 2001): (1) pallor or icterus; (2) lethargy, apnea (respiratory pause lasting more than 10 seconds), bradycardia (heart rate less than 100 per minute in preterms, and less than 80 per minute in term neonates), irritability or seizures; (3) tachypnea (respiratory rate above 70 per minute in preterms, and above 60 per minute in term neonates), retractions or respiratory distress; (4) poor peripheral perfusion, tachycardia (heart rate above 180 beats per minute) or hypotension (blood pressure (BP) below 2SD of mean BP); (5) abdominal distension or vomitus; and (6) fever (central temperature above 37.5°C for at least 4 hours), or temperature instability. Sepsis was defined as a combination of: (1) at least one clinical sign or symptom from each of at least three categories of clinical signs and symptoms, and (2) a positive blood culture. Neonates with negative blood culture who met the two following criteria were classified as having clinical sepsis: (1) at least one clinical sign or symptom from each of at least three categories of clinical signs and symptoms, and (2) elevated I/T-ratio > 0.20 or white blood cell count < 5.0 × 10^3/mm^3 or > 25.0 × 10^3/mm^3 at initial evaluation. Neonates were classified as having pneumonia if they had negative blood culture and met all of the following criteria: (1) radiographic evidence of pneumonia, (2) respiratory signs or symptoms, and (3) elevated I/T-ratio or white blood cell count < 5.0 × 10^3/mm^3 or > 25.0 × 10^3/mm^3 at initial evaluation. Neonates were classified as possibly infected if they had some symptoms or abnormal white blood cell counts, but without fulfilling the criteria of being infected

Blood samples for determining serum leptin, IL-6 and IL-1β were collected from the healthy and the septic neonates before starting treatment, and these investigations were repeated after recovery. IL-6 was measured using an immune-enzymometric assay Kit (IL-6 EASIA, Biosource, Nivelles, Belgium) for the

quantitative measurement of human IL-6 in serum according to the manufacturer instruction statistical analysis. Human IL-1B was assayed by using an immuno-enzymometric assay for the quantitative measurement of human interleukin-1B from Biosource Belgium. Catalogue number KAC1211/KAC1212 according to the manufacture instruction. Intra-assay coefficient of variation 3.4 %. Inter-assay coefficient of variation 4.4%. Leptin was assayed using leptin serum-ELISA as immune-enzymatic assay for quantitative measurement human leptin in serum and plasma from Biosource KAP 2281 according to the manufacturer instructions. Inter-assay 3.6% sensitivity1ng/ml.

Statistical Analysis: A standard t-test was used for comparisons of the characteristics between the two groups. Pearson correlation coefficient was performed to assess the correlation of cytokine levels with serum leptin using SPSS (version 12, SPSS Inc, Chicago, IL). P < 0.05 was considered significant.

RESULTS AND DISCUSSIONS

Results: This study included 14 full term infants (4 males and 10 females) with clinical or proven septicemia; they were followed up until full recovery. They were classified into those under treatment (group I) and after treatment (group II). Fourteen full term healthy infants (6 males and 8 females) were recruited as control group (group III). Clinical and laboratory characteristics of the study groups are shown in table 1.

Serum leptin as well as interleukins IL-6 and IL-1β showed a very high significant increase in their levels on comparing studied cases before and after treatment (group I and II) separately and with the control group (group III) as shown in table (2) and figure (1). Furthermore, there was a very highly significantly decrease of the levels of serum leptin, IL-6 and IL-1β in group II when compared to group I (tables 1& 2 and figure 1).

Interleukins (IL-6 and IL-1β) in the sera correlated significantly with each other before and after treatment. So much so, serum leptin in studied cases before treatment correlated positively with its level after treatment and with IL-6 and IL-1β before and after treatment (figures 2- 7).

Discussion: The results presented above indicate that in our study population IL-6 levels, IL-1β and leptin levels are significantly higher in neonates with sepsis before treatment than in those after treatment or controls. Tumor necrosis factor-α (TNF), interleukin

Table 1: Characteristics of the studied groups

	Group I Cases before treatment	Group III Control	P value
Age (weeks)	39.43±0.76	38.86±0.86	>0.05
Sex (F/M)	10/4	8/6	
Weight (Kg)	3.31±0.53	3.31±0.23	>0.05
Serum IL-6	114.07±55.46	11.13±5.69	< 0.005
Serum IL-1β	43.00±13.32	10.57±2.38	< 0.005
Serum Leptin	3.85±0.69	1.59±0.37	< 0.005

**Table 2:** Comparison between septic neonates before and after treatment

|	Group I Cases before treatment	Group II Cases after treatment	P value
Serum IL-6	114.07±55.46	44.1±18.99	< 0.005
Serum IL-1β	43.00±13.32	20.57±5.06	< 0.005
Serum Leptin	3.85±0.69	2.1±0.36	< 0.05
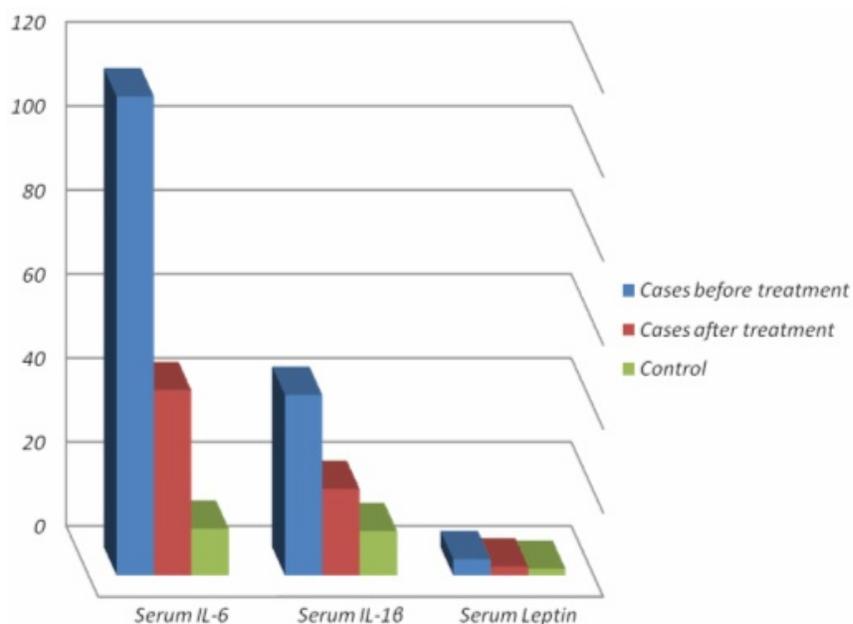
Fig. 1: Comparison between serum levels of IL-6, IL-1B and leptin in studied cases before and after treatment and the control group.

1β (IL-1), and interleukin 6 (IL-6) are well known as important early mediators in the host's initial response to bacterial infection[16,17]. The role of IL-6 in the pathophysiology of septic shock is controversial. High IL-6 levels are associated with acute bacterial infections[18] and endotoxemia[19]. IL-6 increases early during infection probably stimulated by TNF[20]. High levels of IL-6 have been observed in children and in neonates with sepsis[21,22], Messer *et al.,*[23]; Buck *et al.,*[7,24,25] and measurement of IL-6 may be a sensitive diagnostic test[23,7,24]. IL-6 and TNF induce the acute phase response that includes the increase of CRP[20], and TNF enhances the shedding of adhesion molecules from cell membranes[26].

Recent studies reported high IL-6 levels in umbilical cord blood in newborns with early-onset sepsis whose mothers had chorioamnionitis[27,19]. However, even though some newborn infants presented neonatal infection, they had low plasma IL-6 levels[28]. Furthermore, IL-6 can block the transcription of proinflammatory cytokines, however, such as IL-1β and TNF-α (reviewed in reference by Kroemer *et al.,*[29]. Mancuso and colleagues[30] reported data that are compatible with the hypothesis that IL-6 is involved in negative feedback regulation of plasma TNF-α levels in experimental GBS sepsis and that IL-6 pretreatment can increase survival in animal models of GBS-induced
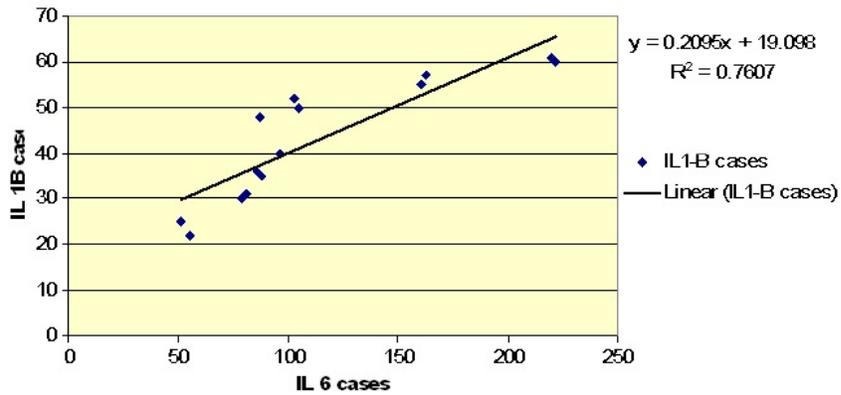
Fig. 2: Correlation between IL-6 and IL-1ß in studied cases
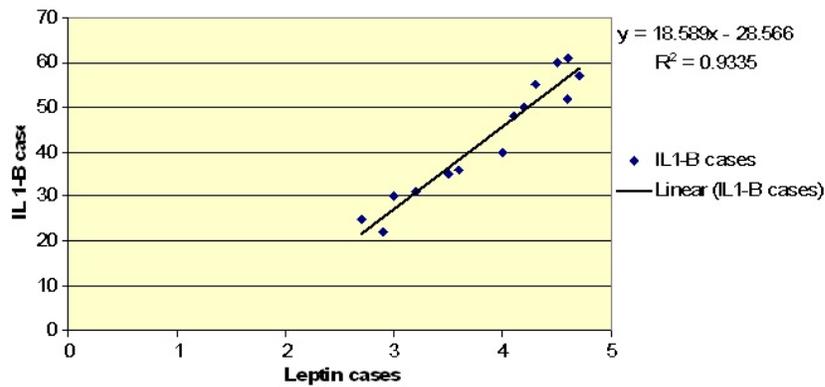
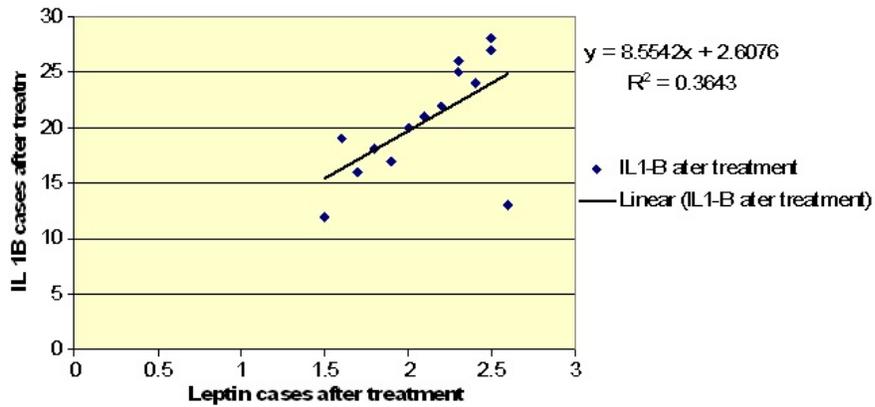**Fig. 3:** Correlation between leptin and IL-1ß in studied cases
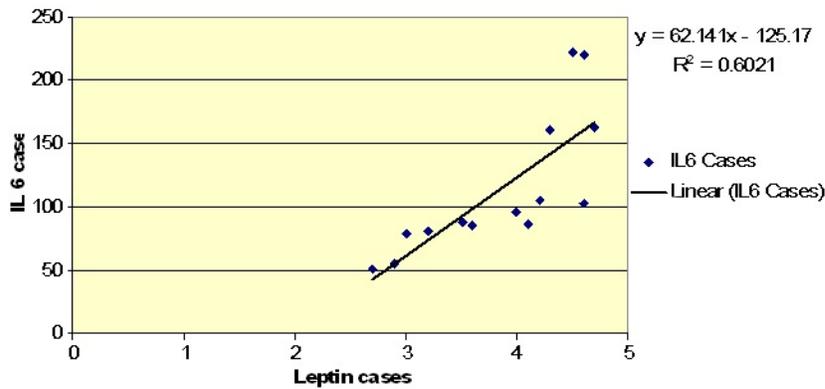
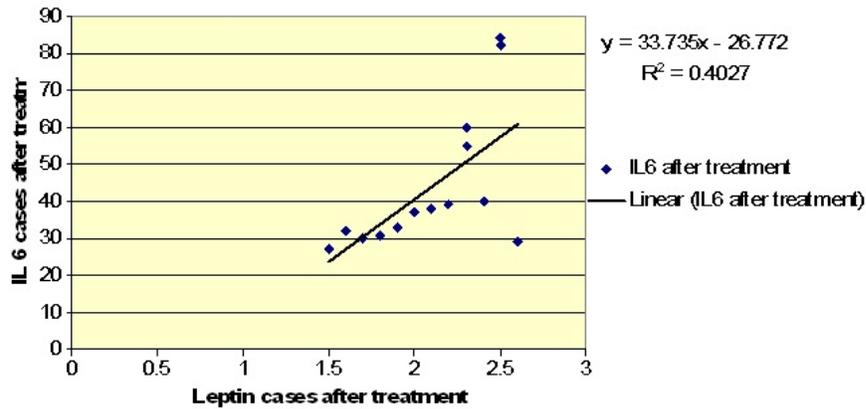**Fig. 4:** Correlation between IL-6 and leptin in studied cases

sepsis. IL-6 is also directly responsible for the induction of acute-phase proteins, many of which have anti-inflammatory properties[31].

IL-1β may be induced by TNF-α and serves to potentiate the effects of TNF-α. In addition, IL-1β acts to stimulate the production of acute-phase reactants by the liver and costimulates T_H1-cell activation[31].

Thus, cytokine analyses are far from being diagnostic for bacterial sepsis, serum levels of IL-6, IL-1 were suggested previously as early markers of neonatal sepsis. In our study, though the increase in the levels of IL-6 and IL-1β before treatment was followed by a significant decrease in their level after treatment yet they failed to reach the control level.

Fig. 5: Correlation between leptin and IL 1-B in studied cases after treatment

Fig. 6: Correlation between leptin and IL-6 in studied cases after treatment
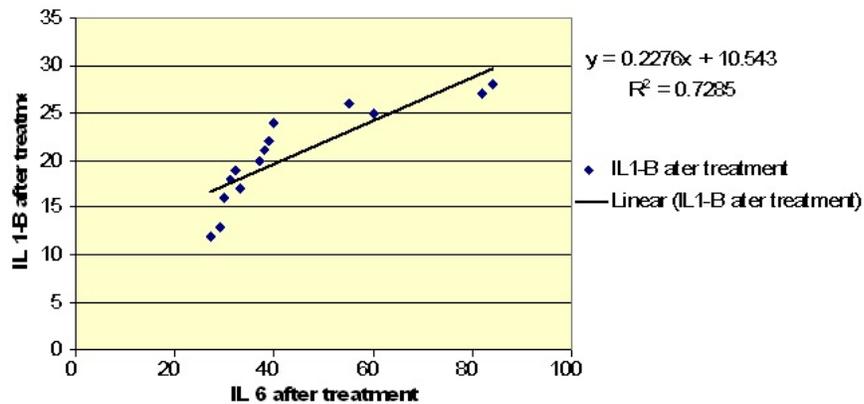
Fig. 7: Correlation between IL-6 and IL 1-B in studied cases after treatment

The purpose of this study was to investigate the effect of sepsis on serum leptin concentration and whether circulating leptin was related to and interleukin-6 (IL-6) and interleukin-1 (IL-1β) release in newborn infants. Serum leptin was measured in 14 neonates with sepsis as soon as sepsis was diagnosed and after recovery and in 14 healthy control infants.

There was a highly significant increase in serum leptin levels between septic and control infants; there was also a highly significant increase in serum leptin levels in septic neonates before than after therapy. A very good relationship between leptin and IL-6; as well as IL-1β before and after therapy was identified. These findings suggest that a role of leptin in acute neonatal

sepsis appears to be likely. Furthermore, serum leptin levels followed IL-6 and Il-1β in their failure to return to control levels

Nevertheless, a role of leptin appears likely, although, it is difficult to document whether this is a major mediating role or a reflection of other more critical endocrine related processes.

REFERENCES

1. Klein, J.O. and S.M. Marcy, 1995. Bacterial sepsis and meningitis. In: J.S. Remington and J.O. Klein, Editors, Infectious diseases of the fetus and the newborn infant, WB Saunders Company, Philadelphia, pp: 835-890.
2. Kocabas, E, A Sarikcioglu, N Aksaray, G Seydaoglu, Y Seyhun and A Yaman, 2007. Role of procalcitonin, C-reactive protein, interleukin-6, interleukin-8 and tumor necrosis factor-alpha in the diagnosis of neonatal sepsis. Turk J Pediatr, 49(1): 7-20.
3. Philip, A.G. and J.R. Hewitt, 1980. Early diagnosis of neonatal sepsis. Pediatrics, 65: 1036-1041.
4. Gerdes, J.S., 1991. Clinicopathologic approach to the diagnosis of neonatal sepsis. Clin Perinatol, 18: 361-81.
5. Mathers, NJ and F Pohlandt, 1987. Diagnostic audit of C-reactive protein in neonatal infection. Eur J Pediatr, 146: 147-151.
6. Hirano, T, 1992. The biology of interleukin-6. Chem Immunol; 51: 153-80.
7. Buck, C, J Bundschu, H Gallati, P Bartmann and F Pohlandt, 1994. Interleukin-6: a sensitive parameter for the early diagnosis of neonatal bacterial infection. Pediatrics, 93: 54-58.
8. Cohen, MC and S Cohen, 1996. Cytokine function: a study in biologic diversity. Am J Clin Pathol; 105: 589-98.
9. Zhang, Y, R Proenca, M Maffei, M Barone, L Leopold and J Friedman, 1994. Positional cloning of the mouse obese gene and its human homologue. Nature., 372: 425-431.
10. Maffei, M, J Halass, E Ravussin, RE Pratlet, GH Lee, Y Zhang, H Fei, S Kim, R Lallone, S Ranganathan, PA Kern and JM Freidman, 1995. Leptin levels in human and rodent: measurements of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med, 1: 1155-1161.
11. Ahima, RS, D Prabakaran, C Mantzoros, D Qu, B Lowell, E Maratos-Flier and JS Flier, 1996. Role of leptin in the neuroendocrine response to fasting. Nature, 382: 250-252.
12. Sarraf, P, RC Frederich, EM Turner, G Ma, NT Jaskowiak, DJ Rivet, JS Flier III, BB Lowell, DL Fraker and HR Alexander, 1997. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J Exp Med, 185: 171-176.
13. Gainsford, T, TA Willson, D Metcalf, E Handman, C McFarlane, A Ng, NA Nicola, WS Alexander and DJ Hilton, 1996. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. Proc Natl Acad Sci USA, 93: 14564-14568.
14. Loffreda, S, S Yang, H Lin, C Karp, M Brengman, D Wang, A Klein, G Bulkley, C Bao, P Noble, M Lane and A Diehl, 1998. Leptin regulates proinflammatory immune responses. FASEB J, 12: 57-65.
15. Lord, GM, G Matarese, JK Howard, RJ Baker, SR Bloom and RI Lechler, 1998. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature, 394: 897-901.
16. Christman, JW, EP Holden and TS Blackwell, 1995. Strategies for blocking the systemic effects of cytokines in the sepsis syndrome. Crit Care Med., 23: 955-963.
17. Abbas, AK, AH Lichtman and JS Pober, 1997. Cellular and Molecular Immunology. 3rd ed. Philadelphia, PA: WB Saunders, 250-277: 342-361.
18. Silveira, RC and RS Procianoy, 1999. Evaluation of interleukin-6, tumor necrosis factor-a and interleukin-1b for early diagnosis of neonatal sepsis. Acta Paediatr, 88: 647-50.
19. Naccasha, N, R Hinson, A Montag, M Ismail, L Bentz and R Mittendorf, 2001. Association between funisitis and elevated interleukin-6 in cord blood. Obstet Gynecol, 97: 220-4.
20. Kilpatrick, L and MC Harris, 1998. Cytokines and the inflammatory response. In: R.A. Polin and W.W. Fox, Editors, Fetal and neonatal physiology, WB Saunders Company, Philadelphia, pp: 1967-1979.
21. de Bont ES, A Martens, J van Raan, G Samson, WP Fetter, A Okken and LH de Leij, 1993. Tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 plasma levels in neonatal sepsis. Pediatr Res, 33: 380-383.
22. de Bont, ES, A Martens, J van Raan, G Samson, WP Fetter, A Okken, LH de Leij and JL Kimpen, 1994. Diagnostic value of plasma levels of tumor necrosis factor alpha (TNF alpha) and interleukin-6 (IL-6) in newborns with sepsis. Acta Paediatr, 83: 696-699.
23. Messer, J, D Eyer, L Donato, H Gallati, J Matis and U Simeoni, 1996. Evaluation of interleukin-6 and soluble receptors of tumor necrosis factor for early diagnosis of neonatal infection. J Pediatr, 129: 574-580.

24. Doellner, H, KJ Arntzen, PE Haereid, S Aag, AM Brubakk and R Austgulen, 1998. Increased serum concentrations of soluble tumor necrosis factor receptors pp: 55 and pp: 75 in early onset neonatal sepsis. Early Hum Dev, 52: 251-261.

25. Panero, LP, N Rossi, G Mancuso, M Stegagno and C Chiesa, 1997. Interleukin 6 in neonates with early and late onset infection. Pediatr Infect Dis J, 16: 370-375.

26. Gearing, AJ and W Newman, 1993. Circulating adhesion molecules in disease. Immunol Today, 14: 506-512.

27. Yoon, BH, R Romero, JS Park, M Kim, SY Oh and CJ Kim, *et al.* 2000. The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin-6 concentration, amniotic fluid infection, and neonatal sepsis. Am J Obstet Gynecol, 183: 1124-9.

28. Reyes, CS, García-Muñoz, D Reyes, G Gonzales, C Dominguez and E Domenech, 2003. Role of cytokines (interleukin-1b, 6, 8, tumor necrosis factor-a, and soluble receptor of interleukin-2) and C-reactive protein in the diagnosis of neonatal sepsis. Acta Paediatr, 92: 221-7.

29. Kroemer, G, I Moreno de Alboran, JA Gonzalo and C Martinez, 1993. Immunoregulation by cytokines. Crit. Rev. Immunol.

30. Mancuso, G, F Tomasello, M Migliardo, D Delfino, J Cochran, JA Cook and G Teti, 1994. Beneficial effects of interleukin-6 in neonatal mouse models of group B streptococcal disease. Infect. Immun., 62: 4997-5002.

31. Dinarello, CA, 1991. The proinflammatory cytokines interleukin-1 and tumor necrosis factor and the treatment of septic shock syndrome. J. Infect. Dis., 163: 1177-1184.