Serum concentrations of interleukin-1 alpha, interleukin-6 and tumor necrosis factor-alpha in neonatal sepsis and meningitis

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ABSTRACT

Objective: To investigate whether serum levels of interleukin-1alpha (IL-1alpha), IL-6, tumor necrosis factor alpha (TNF-alpha), C-reactive protein (CRP) are useful in the diagnosis of neonatal sepsis and meningitis and differentiate them.

Methods: Blood samples were collected from 35 full term neonates with suspected infection who admitted to the Neonatology Unit, Pediatric Department, King Abdul-Aziz University Hospital, Jeddah, Saudi Arabia during January 2002 - June 2003. On the basis of laboratory and bacteriological results, newborns were classified into: sepsis (n=28), meningitis (n=7), and healthy controls (n=16). Sepsis groups were further subdivided according to culture results into: group 1 = proven sepsis (n=6), group 2 = clinical sepsis (n=14), and group 3 = possible-infected (n=8). Serum levels of IL-1alpha, IL-6, TNF-alpha were measured using Enzyme-Linked Immunosorbent Assay while CRP by nephelometer.

Results: In sepsis and meningitis patients, serum levels of CRP ($p<0.01$, $p<0.05$,) and IL-1alpha ($p<0.001$, $p<0.05$) were elevated than controls. C-reactive protein levels elevated in proven sepsis ($p<0.001$) and IL-1alpha elevated in all subgroups of sepsis (groups 1, 2, 3) compared with ($p<0.05$, $p<0.001$, $p<0.01$) controls. Interleukin-6, TNF-alpha showed no significant differences between studied groups. In sepsis and meningitis, IL-1alpha had a highest sensitivity (89%, 86%), and negative predictive values (89% and 93%).

Conclusion: Interleukin-1alpha and CRP increased in neonatal sepsis and meningitis, but cannot differentiate between them. Interleukin-1alpha had a highest sensitivity in prediction of neonatal infection and its assessment may improve accuracy of diagnosis.

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Infectious diseases are an important cause of morbidity and mortality in the neonatal period. Among them, early sepsis is considered to be one of the most threatening conditions.[1] Biological parameters have been evaluated for the early diagnosis of neonatal infection. The most useful ones are white blood cell count (WBC), total number of neutrophils (TN), the immature to total neutrophils ratio (I/T)[1] and the C-reactive protein (CRP).[2] However, the inability of such screening tests to provide definitive guidelines has compelled neonatologists to search for other indicators.[3] An early and accurate diagnosis leading to appropriate therapy would potentially ameliorate the final prognosis of these patients. Therefore, identifying tools for quick detection of early sepsis and meningitis is a highly relevant goal in neonatal medicine. Several cytokines, produced by monocytes, macrophages, and endothelial cells in response to infectious stimuli

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are important proinflammatory mediators in the early phases of the sepsis syndrome.[4] Among cytokines, tumor necrosis factor alpha (TNF-alpha), interleukin (IL)-1ß, IL-6 and IL-8 stimulate the response at several levels.[1] Bacterial sepsis occurs in the neonatal period more frequently than in any other period in life.[5] Susceptibility to bacterial infections is thought to be due to an immature neonatal immune system with a decreased power of antimicrobial defense mechanisms. Reduced secretion of cytokines such as TNF-alpha, IFN-γ, IL-1ß, IL-6, IL-8, and IL-12 has been described in neonatal cells when compared with adult cells.[6] Nevertheless, neonatal monocytes have the capacity to secrete cytokines in vitro,[1] and high levels of TNF-alpha, IL-6, IL-8 have been detected in peripheral blood from septic neonates, suggesting that a mature cytokine response may be established towards term.[7] Several studies have evaluated the role of cytokine determinations as early diagnostic markers in neonatal sepsis.[7,8] Although promising data have been published, problems with invasiveness, response time, and specificity remain to be solved.[9] Despite the considerable number of publications related to cytokines in neonatal infection, there is no agreement about their diagnostic values in these conditions. The objective of this study was to examine the usefulness of determination of levels of IL-1alpha, IL-6 and TNF-alpha in identifying neonatal sepsis and meningitis and differentiation between them.

Methods. This prospective study consists of 35 full term neonates (gestational age ≥37 weeks) consecutively admitted to the Neonatal Unit, Department of Pediatric at University Hospital of King Abdul-Aziz, because of susceptibility of infection, between January 2002 and June 2003. Parental informed consent was obtained for every patient before admission to the study. The Medical Ethics Committee at King Abdul-Aziz Hospital approved the protocol. Data recorded for the study included gender, birth weight, gestational and postnatal ages, mode of delivery, Apgar scores, heart and respiratory rates, blood pressure, rectal temperature, oxygen requirements, O_2 saturation were collected and analyzed. Neonates enrolled in this study were classified according to the final diagnosis into 3 groups: Group 1 controls (n=16), group 2 meningitis (n=7) and group 3 sepsis (n=28). Controls were healthy neonates with a normal postnatal course, without infectious risk factors admitted to well-baby care nursery. Meningitis group was proven by positive culture from cerebrospinal fluid. Causative organisms of meningitis were *Group B streptococcus* and *Escherichia coli*. The sepsis group were further subdivided into 3 subgroups: Proven sepsis (S1, n=6) was defined as a combination of 1) at least one clinical sign or symptom from each of at least 3 categories of clinical signs and symptoms of infection and 2) positive blood culture. Clinical sepsis (S2, n=14) was classified as 1) having negative blood culture 2) positive bacterial culture from elsewhere such as urine, upper airway and 3) met 2 following criteria as described previously:[10,11] a) at least one clinical sign or symptom from each of at least 3 categories of clinical signs and symptoms of infection, and b) elevated ratio immature/total neutrophil (I/T) ratio >0.20 or white blood cell count <5.0 x $10^3/mm^3$ or >25.0 x $10^3/mm^3$ at initial evaluation. Possibly infected (S3, n=8) was neonates had suspected sepsis and abnormal WBC counts, but without fulfilling criteria of being infected and had negative bacterial culture. Final diagnoses in possible infected infants were neonatal hyperbilirubinemia (n=4), transient tachypnea of newborn (n=2), persistent pulmonary hypertension of newborn (n =1) and pneumothorax (n=1). Causative organisms cultured from septic patients included *Staphylococcus aureus*, Coagulase-negative *Staphylococci*, ß-hemolytic *Streptococci*, and *Haemophilus influenzae*. Clinical signs and symptoms of infection as previously described are:[10,11] (i) At least one risk factor for infection; which included rupture of membranes >24 hours, maternal body temperature of >38°C, chorioamnionitis, maternal colonization with group B streptococci. (ii) Signs of respiratory or circulatory dysfunction (tachypnea >60 bpm, recurrent apnea >20 seconds, tachycardia >160 bpm, or bradycardia <100 bpm). (iii) At least one of following clinical symptoms and signs of infection: 1) Pallor or icterus; 2) Lethargy, apnea (respiratory pause lasting more than 10 seconds), bradycardia (heart rate less than 80 per minute in term neonates), irritability or seizures; 3) tachypnea (respiratory rate above 60 per minute in term neonates), retractions or respiratory distress; 4) poor peripheral perfusion, tachycardia (heart rate >180 beats/min) or hypotension (blood pressure below 2SD of mean blood pressure); 5) abdominal distension or vomitus; and 6) fever (central temperature >37.5°C for at least 4 hours), or temperature instability. Evaluation of all newborns was made by 2 clinicians who were blinded to levels of all mediators. None of the infants included had respiratory distress syndrome (RDS), or died, or had intracerebral hemorrhages. Exclusion criteria included prematurely, unstable newborn with respiratory and/or circulatory failure, and infants with neonatal asphyxia or congenital malformations. Blood samples were collected before the start of the antibiotic therapy for blood cultures, WBC counts and its differential and

CRP, second sample was collected in a plain tube, centrifuged (1,500 g for 10 minutes) and stored at –20^{0}C until cytokines were assayed. Cultures from blood, urine and upper airways were obtained from all neonates, and spinal fluid cultures were carried out as part of the septic screening. The CSF samples were immediately centrifuged at 150 g for 10 min, and supernatant was stored at -70^{0}C for further bacteriological and chemistry analyses. In healthy newborns, samples were collected during their stay in the nursery, at time of routine metabolic screening. Complete blood pictures with WBCs differential were carried out on Coulter Counter (Cortland, New York, USA), and CRP was measured by nephelometry BNII (Marbury, Germany). Serum IL-1alpha, IL-6 and TNF-alpha levels were measured using Enzyme-Linked Immunosorbent Assay (Amersham, Biosciences, NJ, USA). The codes RPN of the kits were 2750 for IL-1alpha, 2754 for IL-6 and 2758 for TNF-alpha. According to the manufacturer, the detection limits of the assays for IL-1alpha was 2.0 pg/mL, for IL-6 was 1.0 pg/mL, for TNF-alpha was 5 pg/mL. The intra-assay and inter-assay precision coefficients of variance were <10%. Duplicate measurements were performed for each sample.

Statistical analysis. Values were given as means ± SD (range) using Prism software version 3. The inflammatory mediators were asymmetrically distributed and differences between groups were analyzed using Kruskall- Wallis one-way analysis of variance (ANOVA) and Mann-Whitney U test. Differences between symmetrically distributed variables were tested by one-way ANOVA with Bonferroni's correction. The probability value of <0.05 was considered significant. Sensitivity (true positive/true positive and false negative), specificity (true negative/ true negative and false negative), positive predictive value (PPV) (true positive/ true positive and false positive), and negative predictive value (NPV) (true negative/true negative and false negative) were calculated to analyze IL-1alpha, IL-6, TNF-alpha, and CRP as predictors of neonatal sepsis and meningitis.

Results. The range of gestational age for all participants was 37-39 weeks, and the mean postnatal age did not differ between the groups. There were no differences in gender, birth weight, Apgar score at 5 minutes, or mode of delivery between the studied groups. Red blood cell, platelet and total WBCs counts as well as its differential (neutrophil and lymphocyte) values did not differ significantly between all the studied groups (**Table 1**). Newborns in the entry group of sepsis and meningitis showed higher serum levels of CRP (p<0.001, p<0.05) and IL-1alpha (p<0.001 and p<0.05) compared to controls. Meanwhile, levels IL-6 and TNF-alpha did not show any significant difference in patients with sepsis and meningitis compared to control (**Table 2**). When comparing different categories of sepsis, CRP increased significantly in patients with possible infections; thus, IL-1alpha level significantly increased in all subgroups of sepsis (1st with p<0.05, 2nd p<0.001 and 3rd groups p<0.01 compared to controls) (**Table 3**). No significant correlations were found between the studied cytokines or between the cytokines and the measured hematological parameters (data not shown). The ideal cutoff point for CRP, IL-1alpha, IL-6, and TNF-alpha serum concentrations allows detection of as many true-positive findings as possible (high sensitivity) and with few false-positive results (high specificity). In our study, the optimal cut-off point obtained was 4.02 mg/L for CRP, 9.31 pg/mL for IL-1alpha, 11.43 pg/mL for IL-6, and 29.86 pg/mL for TNF-alpha. At these cut-off points, the PPV for neonatal sepsis and meningitis for CRP were 89% and 75%, for IL-1alpha 89% and 67%, for IL-6 78% and 50% and for TNF-alpha 69% and 20%. The NPV in sepsis and meningitis for CRP were 56% and 93%, for IL-1alpha 81% and 93%, for IL-6 40% and 74% and for TNF-alpha 6%, and 67%. The sensitivity in sepsis and meningitis of CRP were 61% (15 out of 28) and 86% (6 out of 7), for IL-1alpha 89% (26 out of 28) and 86% (6 out of 7), for IL-6 25% (7 out of 28) and 29% (2 out of 7) and for TNF-alpha 32% (9 out of 28) and 14% (1 out of 7). The best sensitivity for sepsis and meningitis achieved by determination of IL-1alpha (**Table 4**).

Discussion. Early diagnosis of neonatal sepsis and appropriate antibiotic treatment can be life-saving and cost-effective. At early onset, the clinical symptoms are nonspecific and difficult to differentiate from transient problems with extra uterine adaptation.[12] Cytokines (IL-1alpha, IL-6 and TNF-alpha) and CRP were measured to identify a set of tests, which can reliably confirm or refute the diagnosis of neonatal sepsis and meningitis. Previously, various WBC counts and CRP had been used to diagnose neonatal sepsis.[11] In this study, WBCs neutrophils and lymphocytes were not differ between studied groups which could be explained by immaturity of immune system of neonates. C-reactive protein is synthesized in the liver in response to TNF-C-reactive protein , IL-ß and IL-6.[1] In consistence with others,[13] this study reported increased in serum levels of CRP in neonates with sepsis and meningitis. We found specificity of CRP in both sepsis and meningitis (88% for both),

Table 1 - Hematological values of the studied neonates.

Parameters	Reference range	Control (n=16)	Sepsis (n=28)	Meningitis (n=7)
Red blood cells (M/UL)				
Mean±SD		4.10 ± 0.803	4.14 ± 0.82	4.34 ± 0.87
Range	4.0 - 5.5	(2.31 - 5.62)	(3.00 - 5.37)	(3.03 - 5.77)
Significance			*p>0.05	*p>0.05
				†p> 0.05
Platelets (K/UL)				
Mean±SD		380.60 ± 107.00	317.80 ± 138.60	404.40 ± 156.00
Range	150 - 450	(169.00 - 522.00)	(110.00 - 598.00)	(258.5 - 636.00)
Significance			*p>0.05	*p>0.05
				†p>0.05
White blood cells (K/UL)				
Mean±SD		8.81 ± 2.67	11.13 ± 3.47	9.55 ± 2.53
Range	5.5 - 15.5	(5.14 - 13.50)	(5.29 - 19.50)	(4.88 - 11.92)
Significance			*p>0.05	*p>0.05
				†p>0.05
Neutrophils (K/UL)				
Mean±SD		2.83 ± 1.12	3.64 ± 2.15	2.74 ± 1.33
Range	1.0 - 8.5	(0.86 - 5.18)	(0.52 - 9.08)	(1.81 - 0.50)
Significance			*p>0.05	*p>0.05
				†p>0.05
Lymphocytes (K/UL)				
Mean±SD		5.58 ± 2.71	5.07 ± 2.91	5.82 ± 1.19
Range	2.0 - 8.0	(1.63 - 12.80)	(1.06 - 12.26)	(4.25 - 7.45)
Significance			*p>0.05	*p>0.05
				†p>0.05

One-way analysis of variance using Bonferroni's Multiple Comparison Test.
*p value was compared to control. †p value was compared to sepsis.

Table 2 - Serum concentrations of C-reactive protein, interleukin -1α, interleukin-6, tumor necrosis factor-alpha in neonates with sepsis and meningitis as well as healthy controls.

Measured parameters	Control (n=16)	Sepsis (n=28)	Meningitis (n=7)
C-reactive protein (mg/l)			
Mean±SD	1.53 ± 2.49	11.50 ± 15.30	5.48 ± 2.71
Range	(0.15 - 8.69)	(0.17 - 46.70)	(0.43 - 9.00)
Significance		*p<0.001	*p<0.05
			†p>0.05
Interleukin-1alpha (pg/ml)			
Mean±SD	7.56 ± 1.75	18.55 ± 12.25	13.71 ± 4.75
Range	(5.00 - 12.00)	(7.00 - 60.00)	(9.00-22.00)
Significance		*p<0.001	*p<0.05
			†p>0.05
Interleukin-6 (pg/ml)			
Mean±SD	6.44 ± 4.99	17.21 ± 45.62	19.00 ± 26.63
Range	(2.00 - 20.00)	(1.00 - 250.00)	(1.00 - 70.00)
Significance		*p>0.05	*p>0.05
			†p>0.05
Tumor necrosis factor - alpha (pg/ml)			
Mean±SD	23.69 ± 6.17	29.00 ± 18.45	20.57 ± 4.28
Range	(18.00 - 40.00)	(10.00 - 90.00)	(18.00 - 30.00)
Significance		*p>0.05	*p>0.05
			†p>0.05

Kruskal-Wallis statistic equations, *p-value was compared to control. †p-value was compared to sepsis.

Table 3 - Serum concentrations of inflammatory mediators in neonates with different grades of septic as well as in healthy controls.

Measured parameters	Control (n=16)	Sepsis groups (n=28)		
		Group 1 (n=6)	Group 2 (n=14)	Group3 (n=8)
C-reactive protein (mg/l)				
Mean±SD	1.53 ± 2.49	4.64 ± 4.36	6.741 ± 9.186	25.81 ± 20.90
Range	(0.15 - 8.69)	(0.17 - 9.5)	(0.17 - 31.50)	(0.60 - 46.70)
Significance		*p>0.05	*p>0.05 †p>0.05	*p<0.001 †p>0.05 ³p>0.05
Interleukin-1alpha (pg/ml)				
Mean±SD	7.56 ± 1.75	15.14 ± 8.07	21.43 ± 15.65	16.50 ± 7.50
Range	(5.00 - 12.0)	(7.00 - 29.00)	(7.00 - 60.00)	(7.00 - 27.00)
Significance		*p<0.05	*p<0.001 †p>0.05	*p<0.01 †p>0.05 ‡p>0.05
Interleukin-6 (pg/ml)				
Mean±SD	6.44 ± 4.99	10.00 ± 6.14	26.07 ± 65.47	8.00 ± 5.10
Range	(2.00 - 20.0)	(2.00 - 18.00)	(1.00 - 250.00)	(5.00 - 18.00)
Significance		*p>0.05	*p>0.05 †p>0.05	*p>0.05 †p>0.05 ‡p>0.05
Tumor necrosis factor-alpha (pg/ml)				
Mean±SD	23.69 ± 6.17	33.00 ± 23.63	24.57 ± 9.56	33.25 ± 25.26
Range	(18.0 - 40.0)	(18.00 - 80.00)	(10.00 - 44.00)	(12.00 - 90.00)
Significance		*p>0.05	*p>0.05 †p>0.05	*p>0.05 †p>0.05 ‡p>0.05

Kruskal-Wallis statistic equations, *p value was compared to control,
†p value was compared to group 1 sepsis, ‡p value was compared to group 2 sepsis.

Table 4 - Comparison of the sensitivity, specificity, positive and negative predictive values of the biochemical tests using the optimal cutoff values in neonatal sepsis and meningitis.

Groups	Measured parameters			
	C- reactive proteins (>4.02 mg/L)	Interleukin-1alpha (>9.31 pg/mL)	Interleukin-6 (>11.43 pg/mL)	Tumor necrosis factor-alpha (>29.86 pg/mL)
Sepsis (n=28) (cut-off point)				
Sensitivity (%)	61	89	25	32
Specificity (%)	88	81	88	75
Positive predictive value (%)	89	89	78	69
Negative predictive value (%)	56	81	40	6
Meningitis (n=7)				
Sensitivity (%)	86	86	29	14
Specificity (%)	88	81	88	75
Positive predictive value (%)	75	67	50	20
Negative predictive value (%)	93	93	74	67

Cut-off values are given in parentheses

with relatively less sensitivity in sepsis (61%) than in meningitis (86%), which is in accordance with previously published findings.[7,13,14] Different studies have undertaken to examine the value of cytokines levels to establish the diagnosis of early sepsis.[15] Previous report[1] showed increase in IL-6 levels early during infection probably stimulated by TNF-α. Meanwhile, IL-6 and TNF-alpha induce acute phase response that includes increase of CRP. The results of the present study showed non-significant increase of either IL-6 or TNF-alpha in neonates with septicemia and meningitis comparing with controls. In contrary to our results, other researchers[7,12,16] reported elevated levels of IL-6 and TNF-alpha in infected term neonates. Meanwhile, other studies[17,18] demonstrated similar or even lower levels of TNF-alpha in infected newborns compared to healthy neonates. It is not clear why our patients did not show raised levels of IL-6 and TNF-alpha, but it is feasible that this finding could be related to immaturity of blood leucocytes described previously in neonates with infectious complications.[17] In this context, other investigators[6] reported that, IL-6 and TNF-alpha were produced rapidly in the early course of neonatal sepsis and peaked on day zero, but their half-lives were short and could fall back to its baseline value within 24 hours.[18] These specific properties of IL-6 and TNF-alpha rendered them useful as a very early alarm hormones, but could not use alone for the diagnosis of infection, because in most circumstances it was uncertain at which stage of infection blood sample was taken for IL-6 and TNF-alpha determination. In contrast, peak concentration of CRP occurred slightly later and it was highly specific for confirming infection. Hence, an abnormally increased CRP with normal plasma IL-6 concentration in sepsis and meningitis neonates suggests that infection has been present for 24 to 48 hours.[19] In disagreement with others,[20] this study showed increase of serum level of IL-1alpha in neonates with sepsis and meningitis compared to controls. When compared the subgroup of sepsis, the possible infected group of patients only showed significant increase compared with controls. This disparity could again be due to the kinetics of these molecules, not completely understood in this early period of life, and because of the inhibitory power of IL-6 on TNF-alpha and IL-1ß (at the transcription level and through stimulation of synthesis of the IL-1ß receptor antagonist and the TNF-alpha soluble receptor).[21] Considering the high mortality and potential morbidity associated with neonatal sepsis, diagnostic tests with high sensitivity and negative predictive value are most desirable because all septic infants have to be identified. The sensitivity of IL-1alpha in the diagnosis of sepsis and meningitis had considerably high level (89% and 86%), however its specificity was low (81% for both). Meanwhile, in sepsis and meningitis the sensitivity of IL-6 and TNF-alpha were low meanwhile their specificity was relatively high. The disparity between the high diagnostic sensitivity reported by Messer et al,[22] for IL-6 plasma levels and neonatal sepsis and ours, could be the result of differences at the time at which blood samples were taken, for IL-6 has a short half-life in plasma.[11] On the other hand, it is worth remembering that IL-6 is the main stimulus involved in the induction of the acute-phase reaction and enhancement of CRP synthesis. Thus, it is expected that the expensive and time-consuming determination of the IL-6 plasma concentration could be replaced by the simpler and more cost effective analysis of CRP levels.

In summary, we conclude that combination of CRP and IL-α is a better diagnostic marker of neonatal sepsis and meningitis than using a separate cytokine as tests. Overlap in IL-1alpha values between cases and controls, and the wide range in its values detract from the strength of IL-1alpha as a predictor of septicemia and meningitis. Furthermore, additional novel markers of neonatal infection should be explored. If our findings are verified in larger studies, a useful test for the early diagnosis of neonatal sepsis and meningitis may be developed.

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