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Study to evaluate the role of TNFα, IL1β, IL6 in diagnosis and severity assessment of neonatal sepsis among term, appropriate for gestational age newborns

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Abstract

Objective: There is no established diagnostic test available to diagnose neonatal sepsis. Tumor necrosis factor α (TNFα), interleukin 1β (IL1β) and interleukin 6 (IL6) showed some promising results in some recent studies in this respect, but their roles in diagnosis and prognosis of neonatal sepsis are not yet conclusive. In this study, we have tried to identify diagnostic and prognostic ability of these biomarkers in neonatal sepsis.

Methods: In this cross-sectional study, blood sample for 3 cytokines were collected at diagnosis of neonatal sepsis fulfilling diagnostic criteria by purposive sampling and values were compared with normal healthy newborns. Each of the cytokines was evaluated separately to identify, whether they can detect neonatal sepsis with at least 70% sensitivity and specificity. At the same time, their prognostic values were also evaluated in terms of disease severity and mortality.

Results: Among twenty normal newborns, standard deviation values of TNFα, IL1β and IL6 were 39.7±21.5 pg/ml, 34.6±20.9 pg/ml and 44.4±33.0 pg/ml, respectively. In sepsis group (n=40), these values were 69.6±26.0 pg/ml, 57.7±29.0 pg/ml and 204.6±169.2 pg/ml, respectively. All these differences were statistically significant ($p<0.05$). IL6 was able to diagnose neonatal sepsis with 80% (95%CI 64.4 to 90.9) sensitivity and 85% (95% CI 62.1 to 96.8) specificity considering a cut off value of 61.8 pg/ml with area under the curve 0.899. This result was better than other two biomarkers.

Conclusion: IL6 may be considered as a good diagnostic tool for neonatal sepsis. None of the biomarkers were able to prognosticate neonatal sepsis.

Keywords: Interleukin 1 beta (IL1β), interleukin 6 (IL6), tumor necrosis factor alpha (TNFα), neonatal sepsis, sepsis prognosis.

Özet: Gestasyonel yaşına göre normal olan term yenidoğanlarda TNFα, IL1β ve IL6'nın neonatal sepsisin tanısı ve şiddetinin değerlendirilmesindeki rolünü incelemeye yönelik çalışma

Amaç: Neonatal sepsis tanısı koymak için mevcut olan kabul görmüş bir tanı testi bulunmamaktadır. Bu bakımdan tümör nekroz faktörü α (TNFα), interlökin 1β (IL1β) ve interlökin 6 (IL6), yakın tarihli bazı çalışmalarda bazı umut verici sonuçlar sergilemiştir; fakat bunların neonatal sepsis tanısı ve prognozundaki rolleri henüz kesinleşmemiştir. Çalışmamızda, bu biyobelirteçlerin neonatal sepsisteki tanılama ve prognostik kapasitelerini tespit etmeye çalıştık.

Yöntem: Bu kesitsel çalışmada, tanılama kriterlerini karşılayan 3 sitokin için neonatal sepsis tanısında amaçlı örnekleme ile kan örnekleri alınmış ve bulunan değerler normal sağlıklı yenidoğanlar ile karşılaştırılmıştır. Her bir sitokin, en az %70 duyarlılık ve özgüllük ile neonatal sepsisi tespit edip edemeyeceklerini belirlemek için ayrı ayrı değerlendirilmiştir. Aynı esnada, hastalık şiddeti ve mortalitesi bakımından prognostik değerleri de değerlendirilmiştir.

Bulgular: Yirmi normal yenidoğan arasında TNFα, IL1β ve IL6'nın ortalama ± standart sapma değerleri sırasıyla 39.7±21.5 pg/ml, 34.6±20.9 pg/ml ve 44.4±33.0 pg/ml idi. Sepsis grubunda (n=40) bu değerler sırasıyla 69.6±26.0 pg/ml, 57.7±29.0 pg/ml ve 204.6±169.2) pg/ml bulundu. Tüm bu farklılıklar istatistiksel olarak anlamlıydı ($p<0.05$). Eğri altındaki alan için 0.899 değeri ve 61.8 pg/ml'lik eşik değer dikkate alındığında, IL6 neonatal sepsisi %80 duyarlılık (%95 güven aralığı 64.4 ila 90.9) ve %85 özgüllük (%95 güven aralığı 62.1 ila 96.8) ile tespit edebildi. Bu sonuç, diğer iki biyobelirteçten daha iyiydi.

Sonuç: IL6, neonatal sepsis için iyi bir tanılama aracı olarak düşünülebilir. Biyobelirteçlerin hiçbiri neonatal sepsisi tahmin edemedi.

Anahtar sözcükler: İnterlökin 1 beta (IL1β), interlökin 6 (IL6), tümör nekroz faktörü alfa (TNFα), neonatal sepsis, sepsis prognozu.

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Introduction

Neonatal sepsis globally is a major cause of mortality and morbidity.[1] South Asia accounts for 3.5 million neonatal sepsis cases per year. Sepsis is the second most common cause of neonatal mortality in India.[2]

The major problem with neonatal sepsis is that it presents with many subtle and nonspecific symptoms. High index of suspicion is necessary to diagnose sepsis early.[3] Severity of illness also varies remarkably among cases. Some of these neonates progress rapidly to severe sepsis and septic shock if not treated early.[4] On the other hand there is significant concern about unnecessary antibiotic use. Frequent use of antibiotics in neonatal intensive care unit (NICU) is a common practice. Multiple and higher generation antibiotics are often used without definite evidence of sepsis and there is emergence of multidrug resistant organism.[5,6]

This problem is possibly aggravated by the fact that there is no single good test available to diagnose and assess severity of sepsis. Blood culture, which is regarded as the gold standard for diagnosis of neonatal sepsis , is time consuming, takes at least 48 to 72 hours and provides a variable yield (8–73%).[7–9]

Different biomarkers, like C-reactive protein, procalcitonin, serum amyloid A, lipopolysaccharide-binding protein, protein biomarkers, cytokines and chemokines, cell-surface antigens have been evaluated for their role in diagnosis of neonatal sepsis.[10] Tumor necrosis factor α (TNFα), interleukin 1β (IL1β), interleukin 6 (IL6) have shown some promises in this respect in recent studies, but their role is not yet conclusive.[11–14] Prognostic value of these biomarkers has been evaluated in very few studies.[15,16] There is also huge variation of study results in different geographical regions.[11,12] Studies from India are limited.[15,17,18] In our study we have tried to evaluate the role of TNFα, IL1β and IL6 in diagnosis of neonatal sepsis and assess if they can predict sepsis severity and mortality.

Methods

This cross sectional study among term, appropriate for gestational age newborns was performed in Sick Newborn Care Unit (SNCU) of a tertiary care teaching Hospital in Eastern India from December 2017 to November 2018. Institutional ethics approval was obtained and informed consent was taken from parents of study subjects.

As there is no valid definition for neonatal sepsis, it was diagnosed based on history, certain risk factors, clinical features and positive sepsis screen result by two independent senior doctors. Relevant history included failure to suck, lethargy, inconsolable cry, abnormal movement, and abnormal skin color. Risk factors for early onset sepsis recorded were low birth weight (<2500 g) or prematurity, febrile illness in the mother with evidence of bacterial infection within 2 weeks prior to delivery, foul smelling and/or meconium stained liquor, rupture of membranes for over 24 hours, single unclean or more than 3 sterile vaginal examination(s) during labor, prolonged labor (sum of 1st and 2nd stage of labor >24 hours), and perinatal asphyxia.[19] Clinical signs of sepsis included the following: apnea, tachypnea (>60/min), nasal flaring, retraction, cyanosis, bradycardia (<100/ min), tachycardia (>180/min), abdominal distention, hypotonia, seizures, and prolonged capillary refilling time (over 2 seconds). The various components of the septic screen were total leukocyte count (<5000/mm^3), absolute neutrophil count (low counts as per Manroe chart[20]), immature to total neutrophil ratio (>0.2), micro-erythrocyte sedimentation rate (>15 mm in 1st hour) and C-reactive protein (>1 mg/dl). If 2 or more parameters of the sepsis screen were positive, then it was considered positive.[7,8,21] Sepsis was diagnosed in presence of any of these clinical features along with positive sepsis screen result. Neonate with more than two risk factors for early onset sepsis with positive sepsis screen was also considered as neonatal sepsis even if asymptomatic.

Term appropriate for gestational age neonates with a diagnosis of sepsis were recruited in the study by purposive sampling. Neonates with congenital malformations, congenital infections associated with the TORCH complex, suspected immunodeficiency were excluded from the study. No preterm or small for gestational age newborn were included in the study. Consecutively all neonates fulfilling these criteria during study period were included in sepsis group. Blood sample for cytokines were also collected from term appropriate for gestational age healthy newborns (controls) from same unit of same hospital during routine blood sampling for neonatal screening of congenital diseases after obtaining informed consent.

Whole blood sample for culture sensitivity and cytokine (TNF-α, IL-1β, IL-10) levels were collected immediately after inclusion in the study and before starting antibiotics. Blood culture was carried out by an automated system (BACT/ALERT® 3D; bioMerieux SA, Marcy l'Etoile, France). Blood sample for cytokines were centrifuged at 2500 rpm for 15 minutes. Separated serum was stored at -80°C. The levels of TNF-α, IL1β, and IL6 were estimated by ELISA as per manufacturer's instruction (Raybiotech Inc., Peachtree Corners, GA, USA). Each cytokines level was measured at 450 nm by ELISA reader (Tecan, Switzerland). Required serum sample for each cytokines were 100 µL. Minimum detectable range was 30 pg/ml (30–6000 pg/ml) for TNFα, 0.3 pg/ml (0.3–100 pg/ml) for IL1β, and 3 pg/ml (3–1000 pg/ml) for IL6.

Newborns were classified as proven sepsis (blood culture positive), suspected sepsis (blood culture-negative) and control (healthy newborns). Sepsis severity was assessed by SNAP II score at the time of diagnosis. A score of ≥40 was considered as severe sepsis.[22] All these babies were prospectively followed up for ultimate outcome: cured/mortality.

Data have been summarized by routine descriptive statistics, namely mean and standard deviation for numerical variables that were normally distributed. Numerical variables were compared between groups by one way analysis of variance (ANOVA), followed by an appropriate post hoc test. Fisher's exact test or Pearson's chi-square test was employed for intergroup comparison of categorical variables. SPSS Statistics version 17 (SPSS Inc., 2008, Chicago, IL, USA) was used for analysis.

We also attempted receiver operating characteristic (ROC) curve analysis, to see whether any of the three biomarkers (TNFα, IL-1β and IL-6) can predicts the occurrence of sepsis with at least 70% sensitivity and specificity. Differences in levels of these biomarkers with respect to disease severity and in survived and mortality group were also assessed.

Results

Among 40 patients with suspected sepsis, 20 were culture positive (proven sepsis) and remaining 20 were culture negative (suspected sepsis). Twenty normal babies were recruited as control. About 50% (n=21/40) of the patients had early onset sepsis. Mean ± standard deviation values of birth weight were 2863.5±464.0 g, 2632.2±304.3 g and 2607.0±271.1 g in normal, suspected sepsis and proven sepsis groups, respectively. Gestational age in these groups was 38.30±1.4 weeks, 38.1±1.0 weeks and 37.8±1.0 weeks, respectively. There was no statistically significant difference between groups. Their baseline characteristics are shown in **Table 1**.

The organisms identified among 20 culture positive patients were Klebsiella (9), Acinetobacter (3), *E. coli* (2), Pseudomonas (2), Enterococcus (2), and *Staphylococcus aureus* (2). Mean ± standard deviation values of leukocyte count, absolute neutrophil count, immature to total neutrophil ratio, erythrocyte sedimentation rate and C-reactive protein levels in sepsis group were 10668.8±7224.9/mm^3, 6007.6±5397.9/mm^3, 0.24±0.16, 13.0±5.8 mm in first hour, 2.2±1.7 mg/dl respectively.

Table 1. Baseline characteristics of three groups.

		Normal	Culture negative sepsis	Culture positive sepsis	p-value
Total number		20	20	20	
Type of sepsis	Early onset	0	10 (50%)	11 (55%)	
	Late onset		10 (50%)	09 (45%)	
Birth weight (g)		2863.5±464.0	2632.2±304.3	2607.0±271.1	0.079
Gestational age (weeks)		38.3±1.4	38.1±1.0	37.8±1.0	0.463
Sex	Male	13 (65.0%)	15 (75.0%)	16 (80.0%)	0.551
	Female	7 (35.0%)	5 (25.0%)	4 (20.0%)	
Mode of delivery	Normal	12 (60.0%)	8 (40.0%)	10 (50.0%)	0.441
	Cesarean section	8 (40.0%)	12 (60.0%)	10 (50.0%)	

Table 2. Comparison of biomarker levels between three study groups.

	Normal	Suspected sepsis	Proven sepsis	p-value for overall comparison	p-value for normal vs. suspected	p-value for normal vs. proven	p-value for suspected vs. proven
TNFα (pg/ml)	39.7±21.5	70.8±26.4	68.4±26.2	<0.001	0.01	0.002	0.948
IL1β (pg/ml)	34.6±20.9	53.5±23.5	62.0±33.7	0.006	0.073	0.005	0.575
IL6 (pg/ml)	44.4±33.0	185.0±166.1	224.2±174.1	<0.001	0.007	<0.001	0.652

Values of TNFα, IL1β and IL6 in different groups were illustrated in **Table 2** and **Fig. 1.** Irrespective of blood culture report, there was statistically significant difference in the levels of all 3 cytokines between sepsis group and normal newborns (p<0.05). However, there was no statistically significant difference of plasma cytokine levels between suspected and proven sepsis groups (p>0.05).

Utility of each of the biomarkers to diagnose sepsis (both suspected and proven) assessed by ROC analysis showed area under the curve (AUC) to be 0.814, 0.740 and 0.899, respectively for TNFα, IL1β and IL6. Criterion value assessed from ROC curve were >36.8 pg/ml for TNFα (sensitivity 95%, specificity 55%), >56.5 pg/ml (sensitivity 50%, specificity 95%) for IL1β and >61.8 pg/ml (sensitivity 80%, specificity 85%) for IL6. Thus only IL6 offered satisfactory sensitivity and specificity towards diagnosing sepsis in the newborns (**Table 3** and **Fig. 2**).

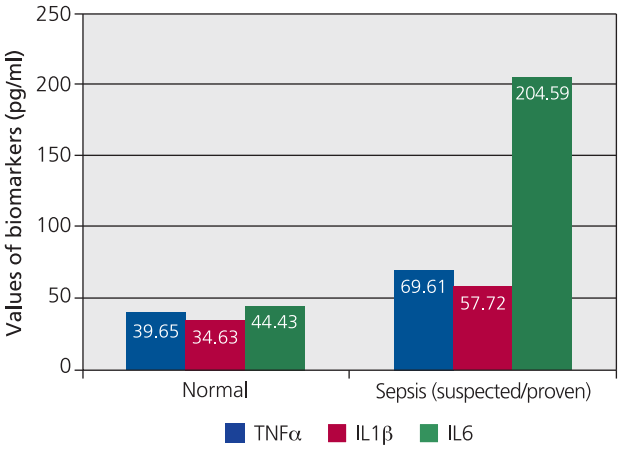

Fig. 1. Bar diagram showing mean difference in biomarkers between normal newborns and newborns with sepsis.

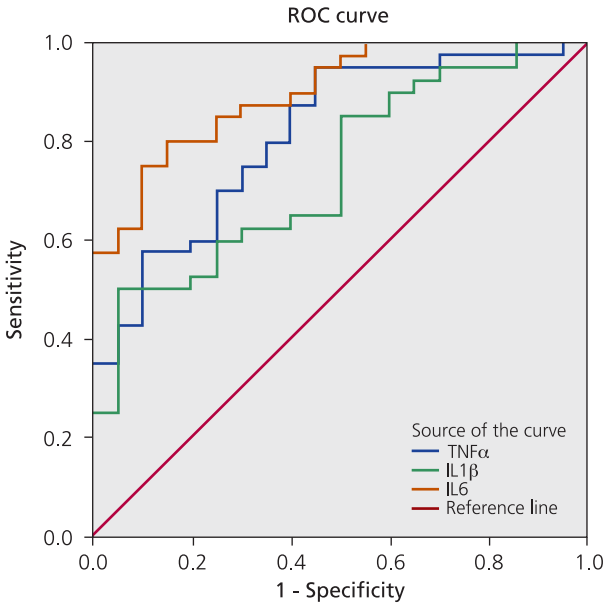

Fig. 2. ROC curve of three cytokines with reference line.

Table 3. Receiver operating characteristic (ROC) curve analysis to show performance of three biomarkers in diagnosing neonatal sepsis.

	TNFα	IL1β	IL6
Area under the ROC curve (AUC) with 95% CI	0.814 (0.692 to 0.903)	0.740 (0.611 to 0.845)	0.899 (0.793 to 0.962)
Criterion (cut-off) value (pg/ml)	>36.8	>56.5	>61.8
Sensitivity (%) with 95% CI	95.0 (83.1 to 99.4)	50.0 (33.8 to 66.2)	80.0 (64.4 to 90.9)
Specificity (%) with 95% CI	55.0 (31.5 to 76.9)	95.0 (75.1 to 99.9)	85.0 (62.1 to 96.8)

Table 4. Values of biomarkers in different outcome groups.

	Disease severity assessed by SNAP II score SNAP II score (≥40 indicates severe sepsis)			Survival/mortality		
	Severe sepsis (n=15)	Non-severe sepsis (n=25)	p- value	Survived (n=16)	Not survived (n=24)	p- value
TNFα (pg/ml)	72.2±22.9	65.3±30.8	0.427	72.1±23.2	65.9±30.1	0.465
IL1β (pg/ml)	49.8±22.3	62.5±31.8	0.181	62.6±31.0	50.4±24.9	0.195
IL6 (pg/ml)	237.6±194.5	184.8±152.8	0.345	188.6±155.5	228.7±190.4	0.470

There were no statistically significant differences in any of the biomarkers' levels between severe sepsis and non-severe sepsis groups. There were also no statistically significant findings in relation to any of the three biomarkers with respect to mortality prediction (**Table 4**).

Discussion

In our study we found that values of all 3 biomarkers namely TNFα, IL1β and IL6, were significantly high in neonate with sepsis as compared to healthy ones. IL6 was found to have diagnostic ability for neonatal sepsis with reasonable sensitivity and specificity. However, none of the biomarkers were able to prognosticate sepsis severity in terms of treatment support and mortality.

Studies from different part of the world have shown some important role of these biomarkers in diagnosis of neonatal sepsis, but there is non-uniformity in sensitivity, specificity and cut off values between studies. In a recent meta-analysis on TNFα as a diagnostic marker of neonatal sepsis by Bokun et al.,[11] has reported that studies done in Northern hemisphere had a pooled sensitivity of 84.0% and specificity was 83.3% while that for Southern hemisphere was 68.0% and 88.5% respectively for diagnosis of late onset neonatal sepsis. For diagnosis of early onset sepsis pooled sensitivity was 66.1% and specificity 75.6%.[11] In our study we have found TNFα had 95% sensitivity and 55% specificity to diagnose neonatal sepsis irrespective of type of sepsis.

Atici et al.[23] in their study found that IL1β level diminishes in neonatal sepsis while in other studies it was elevated.[14,24] IL1β was found to have a sensitivity of 27% and specificity of 70% to diagnose neonatal sepsis in the study by Ayazi et al.[24] On the other hand we found that IL1β levels increased in neonatal sepsis with sensitivity of 50% and specificity of 95%.

IL6 has been the most extensively studied interleukin till date. It has sensitivity of 71–100% and specificity of 47–95% to diagnose neonatal sepsis in different studies with different cut off values (10–100 pg/ml).[12] IL6 has a very short half-life and hence, it shows decline in sensitivity within 24–48 hours.[25] Some studies that have explored both TNFα and IL6 showed that, as a single biomarker diagnostic tool, TNFα was better than IL6.[17,26] TNFα has showed 60% sensitivity and 100% specificity, in combination with IL6 levels, for the diagnosis of sepsis in study by Debont et al.[27] In our study, we found a good sensitivity for TNFα but with a low specificity, while IL1β had a good specificity but with poor sensitivity. IL6 was found to have reasonable sensitivity (80%) and specificity (86%) with maximum AUC (0.899). So it may be considered as the best single biomarker among the three for diagnosis of neonatal sepsis.

There are few studies that have highlighted the prognostic relation of elevations of these biomarkers and severity of sepsis. Girardin et al.[16] had shown that serum TNFα levels may have a direct correlation with the severity of sepsis and the mortality rate during the development of sepsis in newborns at risk for infections. Studies on adults with sepsis have shown increasing IL6 level to be associated with higher mortality rates.[28] Even after extensive search of published literature, no data on prognostic value of IL1β could be found. However, our analysis of newborns with sepsis did not reveal any relation of elevated biomarker levels with severity of sepsis.

Previous studies have demonstrated difference in level of biomarkers in acute and post-acute phase, and also with treatment.[14,29] However, ours being a cross

sectional study where serial measurements were not carried out, and therefore changes in the level of biomarkers with course of disease were not identified. Although prematurity and small for gestational age (SGA) are known risk factors for neonatal sepsis, they were not included in the study for the possibility of variation in biomarkers' levels in these risk factors. As this study was done only among term AGA babies, the role of these biomarkers among preterm and small for gestational age newborns are not determined. Further study in these groups of neonate is needed, as they are more vulnerable population.

Conclusion

Although TNFα, IL1β and IL6 were not able to prognosticate neonatal sepsis, they had a good role in diagnosis of neonatal sepsis among term appropriate for gestational age neonates. All these biomarkers were significantly elevated in neonatal sepsis and IL6 may have diagnostic utility with reasonable sensitivity (80%) and specificity (85%).

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Compliance with Ethical Standards: The authors stated that the standards regarding research and publication ethics, the Personal Data Protection Law and the copyright regulations applicable to intellectual and artistic works are complied with and there is no conflict of interest.

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