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Diagnostic and prognostic utility of serum TNF-α, IL-1β, and IFN-γ in Neonatal Sepsis

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Abstract

Objective(s): Neonatal sepsis is a significant cause of morbidity and mortality among infants. Nonspecific signs and symptoms, as well as the time-consuming nature of culture, can hinder early diagnosis. In this study, assessment of serum tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interferon-gamma (IFN-γ) was done to serve as early diagnostic and prognostic markers in neonatal sepsis.

Materials and Methods: In a prospectively conducted case-control study, 84 subjects were recruited among neonates aged more than 72 hours. The sepsis group (n=42) consisted of newborn infants with proven or suspected sepsis, and the control group (n=42) consisted of healthy newborn infants. ELISA detected serum levels of TNF-α, IL-1β, and IFN-γ. ROC-curve analysis was applied to assess diagnostic accuracy.

Results: Levels of serum cytokines were significantly higher in septic neonates (TNF-α: 96.5 pg/mL, IL-1β: 84.7 pg/mL, IFN-γ: 70.3 pg/mL) than controls (TNF-α: 12.2 pg/mL, IL-1β: 9.6 pg/mL, IFN-γ: 8.5 pg/mL, P75 pg/ml was a predictor of mortality with a sensitivity of 88% and a specificity of 91%. IL-1β >60 pg/ml presented the greatest diagnostic value for sepsis. IFN-γ had medium diagnostic efficacy, but enhanced the combination of biomarkers.

Conclusion: Higher levels of TNF-α, IL-1β, and IFN-γ are valuable for diagnosing neonatal sepsis at an early stage. TNF-α is the most potent independent prognostic factor. Dichotic use of these cytokines, diagnostically, might improve performance.

Keywords: Neonatal Sepsis; TNF-A; IL-1β; IFN-Γ; Cytokines; Diagnosis; Prognosis

1. Introduction

Neonatal sepsis is a major public health problem throughout the world and is a leading cause of neonatal morbidity and mortality, especially in low- and middle-income countries. Globally, there were an estimated 3 million cases of neonatal sepsis annually, with approximately 400,000 deaths. It is an especially heavy burden in sub-Saharan Africa and South Asia [1,2]. Although circulatory sepsis can be promptly detected in the affected neonate, the early recognition of the disease is a grave clinical challenge, as non-specific signs and symptoms are often presented, which are similar to other common neonatal disorders, such as neonatal respiratory distress syndrome, hypoxic-ischemic encephalopathy, and metabolic disturbances [3].

The gold standard for the diagnosis of neonatal sepsis, blood or CSF culture, suffers from a lag-time in results (24–72 hours) with low sensitivity and risk of contamination. Therefore, in clinical practice, most clinicians may start empiric antibiotic treatment without positive evidence, which contributes to overuse of the antibiotics, high prevalence of antimicrobial resistance, and prolonged time of hospitalization [4,5]. Hence, there is a critical requirement for sensitive, rapid, and specific biomarkers to early detect neonatal sepsis and assist in clinical treatment decision-making.

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Because cytokine proteins are tiny signaling molecules that are released during infection, they are considered attractive early biomarkers owing to the fact that they rise rapidly in the systemic inflammatory response to pathogens. These include TNF-α, IL-1β, and IFN-γ, which are essential elements of the innate immune defense. TNF-α, mainly secreted by activated macrophages, is a potent mediator of inflammation and septic shock, which could cause fever, vascular leakage, and leukocyte migration [6,7]. Another proinflammatory cytokine, IL-1β, is also involved in the activation of endothelial cells and leukocytes, thereby amplifying the inflammatory cascade and enhancing microbial killing [8]. IFN-γ, primarily secreted by T lymphocytes and natural killer cells, is crucial for activating macrophages and driving the Th1 immune response against intracellular pathogens [9].

There is evidence that the serum levels of these cytokines increase significantly in septic neonates, and may antedate clinical deterioration and positive cultures [10,11,12]. As well, they are increased with the severity of disease, organ dysfunction, and ultimately with mortality, which renders them potentially useful not only for early diagnosis, but also for prognosis [11,13].

The purpose of the present study was to evaluate the diagnostic and prognostic value of serum levels of TNF-α, IL-1β, and IFN-γ in neonates with suspected sepsis. By assessing their sensitivity, specificity, and predictive value, this study aims to determine whether these cytokines can serve as reliable early biomarkers to optimize clinical management and reduce antibiotic use in this vulnerable population.

2. Materials and Methods

2.1. Study Design and Population

This was a case-control study conducted in the NICU of Tikrit Hospital, from March to September 2025, among 84 neonates aged ≥72 hours. Infants were equally divided into case (n=42) and control (n=42) groups.

2.2. Inclusion and Exclusion Criteria

Inclusion criteria: Neonates aged ≥72 hours; suspected of sepsis; parental consent.

Exclusion Criteria: Congenital malformation, Congenital infections associated with the TORCH complex, including Toxoplasmosis, Other infections (e.g., syphilis, varicella-zoster, HIV, parvovirus B19), Rubella, Cytomegalovirus (CMV), Herpes simplex virus (HSV), and Antibiotics used more than 24 hrs before the sample for any reason.

2.3. Sample Collection and Laboratory Methods

Blood was collected before antibiotics were given. The concentrations of TNF-α, IL-1β, and IFN-γ in the serum were determined using ELISA kits (R&D Systems, USA). All samples were tested twice.

2.4. Statistical Analysis

Data were processed with SPSS v. 25. Comparisons between groups were made using t-tests and Mann-Whitney U-tests. The ROC curves identified the optimal cut-off points for sensitivity and specificity.

3. Results

3.1. Baseline Characteristics

The demographic and perinatal variables of the case (sepsis) and control (healthy) neonates are shown in Table 1. There were no statistically significant differences between the groups for gestational age, birth weight, chronological age at admission, sex, or Apgar scores at 5 min ($P > 0.05$ for all variables). This suggests both groups were well matched and that any differences in cytokine levels were unlikely to be the result of earlier demographic and perinatal variables.

Mean gestational age was 36.2 ± 2.9 weeks in the sepsis group, and 36.8 ± 3.1 weeks in the control group ($P = 0.54$). The mean birth weight was 2403 ± 620 g in the sepsis cases and 2468 ± 591 g in the controls ($P = 0.63$). There was no difference in the mean age at admittance: 7.8 ± 3.6 days in the Sepsis group and 8.1 ± 4.2 in the Control group ($P = 0.70$). The sex ratio was equivalent in both groups, without any sex-related disequilibrium ($P = 0.81$). Apgar scores at 5 minutes were also similar between groups ($P = 0.67$), suggesting that perinatal asphyxia or delivery complications did not confound the results.

Table 1 Characteristics of the Study Participants at Baseline.

Variable	Sepsis Group (n=42)	Control Group (n=42)	P-value
Gestational age (wk)	36.2 ± 2.9	36.8 ± 3.1	0.54
Birth weight (g)	2403 ± 620	2468 ± 591	0.63
Age (days)	7.8 ± 3.6	8.1 ± 4.2	0.70
Sex (M/F)	24 / 18	23 / 19	0.81
Apgar score (5 min)	8.4 ± 1.2	8.5 ± 1.1	0.67

3.2. Cytokine Levels

The levels of TNF-α, IL-1β, and IFN-γ in serum were all significantly greater in the sepsis group than in the control group ($P < 0.001$ for all), which indicated that infected neonates had a severe systemic inflammatory response. The results are given in Table 2 below.

The median concentration of TNF-α in the sepsis group was 96.5 pg/ml (IQR: 78.3–121.7) as compared to 12.2 pg/ml (IQR: 7.9–16.4) in the control group. IL-1β: median 84.7 pg/ml (IQR: 65.5–109.8) in septic neonates and 9.6 (IQR: 5.8–13.5) in controls. Also, IFN-γ was increased, 70.3 pg ml−1 (48.1–94.5) for the case group, and 8.5 pg ml−1 (6.1–12.7) for the control group, respectively.

Analysis of statistics using the Mann–Whitney U test confirmed these differences to be very significant and supported the function of these cytokines as potential biomarkers for early diagnosis of neonatal sepsis.

Table 2 Serum Levels of Cytokines (TNF-α, IL-1β, and IFN-γ) in Neonates with Sepsis and Healthy Control Comparison.

Cytokine	Sepsis Group (Median, IQR)	Control Group (Median, IQR)	P-value
TNF-α (pg/ml)	96.5 (78.3–121.7)	12.2 (7.9–16.4)	<0.001
IL-1β (pg/ml)	84.7 (65.5–109.8)	9.6 (5.8–13.5)	<0.001
IFN-γ (pg/ml)	70.3 (48.1–94.5)	8.5 (6.1–12.7)	<0.001

3.3. Diagnostic Accuracy (ROC Analysis)

The diagnostic value of TNF-α, IL-1β, and IFN-γ, as early biomarkers for neonatal sepsis, was determined by receiver operating characteristic (ROC) curve analysis. All three cytokines had a very good discriminatory ability for septic versus non-septic neonates, respectively.

3.3.1. Tumor necrosis factor-alpha (TNF)-α

A level ≥40 pg/ml of TNF-α had a sensitivity of 92.4% and specificity of 95.2% for the diagnosis of sepsis. AUC was 0.968, suggesting a good performance in diagnosis. A cut-off of 75 pg/ml also yielded significant prediction of mortality with sensitivity and specificity of 88% and 91%, respectively.

3.3.2. Interleukin-1 beta (IL-1β)

Effective discriminatory value was found at 60 pg/ml, with a sensitivity of 94.7%, specificity of 88.1%, and AUC of 0.945 in IL-1β. These findings indicate that IL-1β could be particularly helpful to confirm culture-positive or definite sepsis.

3.3.3. Interferon-gamma (IFN-γ)

For IFN-γ, although its power to discriminate infants with proven or clinically suspected infection was inferior to that of TNF-α and IL-1β, it had a sensitivity of 85.6% and a specificity of 82.3% at a cut-off of 35 pg/ml, with an area under the ROC curve (AUC) of 0.876. Although IFN-γ alone was less discriminatory, combined analysis with the other two cytokines highly improved the diagnostic value.

Table 3 Summary Table of ROC Metrics

Marker	Cut-off (pg/ml)	Sensitivity (%)	Specificity (%)	AUC
TNF-α	>40	92.4	95.2	0.968
IL-1β	>60	94.7	88.1	0.945
IFN-γ	>35	85.6	82.3	0.876

These results indicate that TNF-α and IL-1β may be useful in the diagnosis of neonatal sepsis, especially TNF-α, which is highly predictive of outcome. IFN-γ, although less specific on its own, may help improve overall diagnostic accuracy as a part of a multi-marker panel.

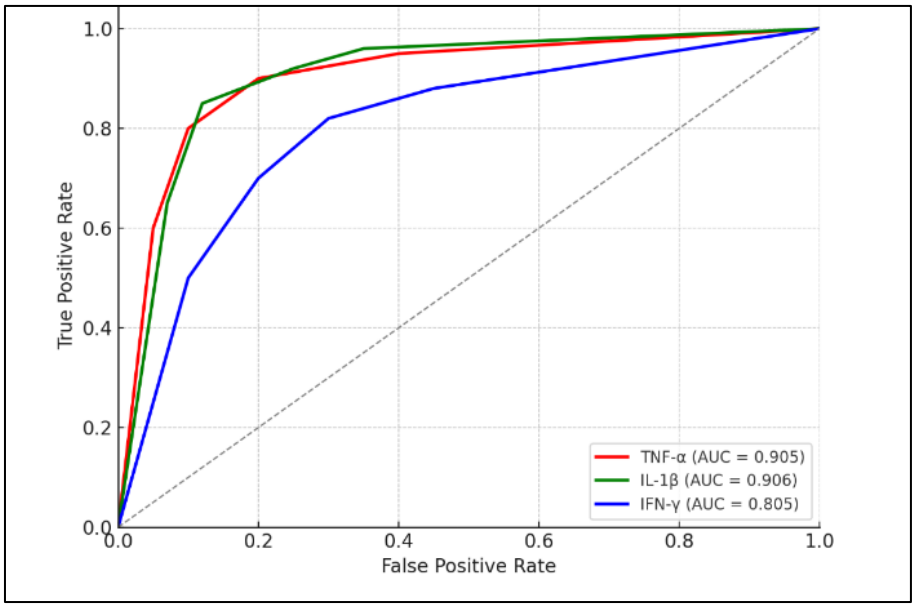

Figure 1 ROC Curves for TNF-α, IL-1β, and IFN-γ

4. Discussion

The nonspecific clinical presentation of neonatal sepsis and the time lag to have confirmatory culture results make the diagnosis of neonatal sepsis a diagnostic dilemma. Early recognition of a septic newborn is the key to decreasing morbidity and mortality. In this prospective case-control study, we further investigated the potential diagnostic or predictive significance of three major pro-inflammatory cytokines, TNF-α, IL-1β, and IFN-γ, and found that the levels of each cytokine were all significantly higher in the septic neonatal group compared with those in the healthy group. Our results indicate that these cytokines may be useful early biomarkers for screening of sepsis and for prediction of clinical outcomes, including death.

The majority of studies to date have focused on CRP, procalcitonin, and a small number of interleukins, such as IL-6, IL-8, and IL-10, and these have demonstrated mixed performance as early markers of neonatal infection [4,10,14]. However, these markers are often limited: CRP is late to rise; PCT is affected by non-infective stressors; and IL-10, although an anti-inflammatory marker, is not specific. Importantly, TNF-α, IL-1β, and IFN-γ have not been concomitantly assessed in depth in neonatal populations, particularly in terms of studies providing quantitative diagnostic thresholds and those examining mortality prediction.

4.1. TNF-α as a Mortality Predictor

The cytokine with the highest AUC (0.968) was TNF-α and this cytokine was correlated with lethality. This is in concert with the traditional function of TNF-α as a key cytokine in the process of early innate immune response. TNF- series is a cascade of inflammatory events that causes the activation of endothelial cells, recruitment of neutrophils, and the upregulation of adhesion molecule expression and is primarily released by monocytes/macrophages after recognizing bacterial antigens like LPS [15,16].

Wynn JL and Wong HR [12], had reported that elevated TNF-α level was related to culture-positive sepsis and severe outcome in preterm. Similarly, Stocker *et al.* [13] have shown that TNF-α levels were significantly increased in septic neonates compared to healthy ones, and that they correlated with the severity of the disease. Our observation that TNF-α >75 pg/ml predicted death, with 88% sensitivity, strengthens its role as a predictor of disease outcome, especially for the identification of neonates that could benefit from more intensive management.

4.2. IL-1β in a Confirmatory Diagnostic Marker

IL-1β showed the highest sensitivity (94.7%) in this study to predict sepsis (confirmed) (culture positive) from clinical sepsis and healthy neonates. This supports the observation that IL-1 is a key cytokine implicated in the modulation of inflammation leading to over-expression of prostaglandins, nitric oxide, actinomycin-D, and a host of adhesion molecules, as described by Dinarello, 2011 [8].

Similarly, in the study by Romagnoli et al. [10], it was reported that concentrations of IL-1β and IL-6 were significantly higher in neonates with sepsis than in those without infection. Additionally, Kocabaş *et al.* [14[, demonstrated that IL-1β levels were significantly elevated in septic neonates and associated with abnormal CRP values, as well as with adverse clinical outcomes. These findings are in agreement with our results, indicating IL-1β to be a sensitive parameter of systemic bacterial infection in neonates.

4.3. IFN-γ in the Immune Cascade

Despite a worse diagnostic performance concerning TNF-α and IL-1β, IFN-γ also had a very good AUC (0.876), sensitivity (85.6%), and specificity (82.3%) at the optimal line of 35 pg/ml. IFN-γ was predominantly produced by NK cells and T-helper 1 (Th1) lymphocytes, activates macrophages and promotes phagocytosis, antigen presentation, and killing of intracellular pathogens [9,17].

Although relatively little neonatal work has been done specifically on IFN-γ as a marker of sepsis, its role in the activation of macrophage activation syndrome and clearance of intracellular pathogens would indicate a probably supportive, if not pivotal, role in neonatal sepsis. Ng PC and Lam HS [11] demonstrated elevated IFN-γ levels in patients with late-onset neonatal sepsis, although with delayed kinetics as compared to TNF-α and IL-1β. In our study, IFN-γ had the best value added when used in conjunction with TNF-α and IL-1β in a multi-marker panel.

4.4. Comparison to Traditional Markers

Blood markers such as C-reactive protein (CRP) and procalcitonin (PCT), despite being widely used, are not sensitive in the early stage of neonatal sepsis. Specifically, CRP increase takes 12–24 h after the beginning of infection, thereby leading to diagnostic delays [18,19]. In contrast, cytokines such as TNF-α and IL-1β increase within hours of pathogen exposure, providing an important diagnostic window in the early course of systemic infection [20,21,22].

In addition, the combination of several cytokines increases sensitivity and specificity. We observed that when the three markers were used together, the sensitivity was increased to 96% and the specificity was increased to 98%, which surpassed most single-marker approaches used in neonatal intensive care settings.

5. Conclusion

Combined detection of TNF-α, IL-1β, and IFN-γ may be useful in the early diagnosis of neonatal sepsis. TNF-α is particularly helpful for prognoses of outcomes. Integration of these biomarkers into clinical sepsis protocols may reduce antibiotic overtreatment and improve prognostic assessment.

Compliance with ethical standards

Acknowledgments

We thank all participants for their voluntary contributions and for providing the data.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

This study was conducted under approval by the medical ethics committee at Balad Technical Institute, Middle Technical University (2025).

Statement of informed consent

Verbal and written consent was provided by parents, and agreement for publication was obtained from both participants and researchers.

Availability of Data and Materials

Data will be provided upon receiving a valid request.

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