ORIGINAL ARTICLE

Evaluation of hematological markers (TLC, neutrophil count) and CRP protein in early diagnosis of neonatal sepsis, taking blood culture as gold standard

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ABSTRACT

Background: Neonatal sepsis is one of the major causes of neonatal mortality and morbidity. This study was done to determine the accuracy of TLC and Neutrophil count for early diagnosis of neonatal sepsis and to determine the diagnostic accuracy of C-Reactive protein (CRP) for early diagnosis of Neonatal sepsis.

Material and Methods: It was a cross-sectional study performed in Microbiology department, of a tertiary care Hospital, Lahore. A total of 241 blood samples were collected in the blood culture bottles, incubated and inoculated on blood and MacConkey agar plates. Final identification was performed using API and VITEK2. Antimicrobial susceptibility testing (AST) was performed according to CLSI 2024.

Results: Out of total 241 study subjects, 150(62%) were males and 91(38%) were females. 49 (20.3%) blood culture were positive. Among the isolated microorganisms, *Acinetobacter baumannii* 8(17%), *Acinetobacter jhonsonii* 3(6%), *coagulase negative Staphylococci* 15(31%), *Methicillin resistant Staphylococci* 3(6%), *Enterococcus faecalis* 3(6%), *Klebsiella pneumoniae* 8(16%), *Listeria monocytogenes* 1(2%), *Pseudomonas aeruginosa* 2(4%), and *Serratia marcescens* 6(12%) Seen. Majority of the gram-positive bacteria were resistant against penicillin, and sensitive to vancomycin and linezolid. Whereas, the gram-negative bacteria are most resistant to ampicillin, least resistant noted against carbapenems.

Conclusion: The present study aims to identify the role of hematological indicators as TLC, DLC and CRP, as early indicator of neonatal sepsis. The results of the hematological markers and serum CRP received in a much less time as compared to the blood culture that gives results after the delay of 4 days.

Keywords: Neonatal sepsis, TLC, CRP, Rapid diagnosis

BACKGROUND

Neonatal sepsis is the condition with the symptoms and signs of the systemic infection through the early 4 weeks of life. The early-onset neonatal sepsis (EONS) is sepsis during early 72 hours of life whereas late-onset neonatal sepsis (LONS) happens after 72 hours.[1] While blood culture is regarded as the gold standard for diagnosing neonatal septicemia, there is a significant delay of four days in receiving the results. This extended timeframe poses a substantial risk as it coincides with a critical period during which many neonates may not survive.[2]

Early-onset neonatal sepsis occurs either in uterine life through placental transmission or, more frequently, when microorganisms ascend from the vaginal environment into the uterus after rupture of membrane. Furthermore, the neonate can acquire infection when encountered to possibly harmful microorganisms while the transit through the birth canal.[3] Chorioamnionitis is characterized by inflammation of the fetal membranes, typically caused by microbial invasion of the amniotic fluid, often occurring due to lengthy estrangement of the Chorio-amniotic portion. Diagnosis of chorioamnionitis may involve observing maternal signs and symptoms such as increased temperature, leukocytosis, dirty or malodorous discharge, and abdominal pain, along with other indicators, with increased heart rate being the most prevalent.[4]

According to the data generated by Jehan *et al*., two-thirds of the neonatal deaths in the world happen in 10 countries, typically Asia. Pakistan is at three number in these. Pakistan contributes to 7% of neonatal deaths worldwide, while the attainment of Millennium Development Goals (MDGs) 4 and 5 remains unmet. Globally, infections (36%), premature birth (28%), and asphyxia at birth (23%) collectively constitute 87% of neonatal fatalities. Preterm birth and birth asphyxia are

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This article can be cited as: Khan ZA, Akhtar A. Evaluation of hematological markers (TLC, neutrophil count) and CRP protein in early diagnosis of neonatal sepsis, taking blood culture as gold standard. Infect Dis J Pak. 2025; 34(1): 40-45.

DOI: https://doi.org/10.61529/idjp.v34i1.340

Receiving date: **24 Aug 2024** Acceptance Date: **21 Jan 2025**
Revision date: **31 Dec 2024** Publication Date: **30 Mar 2025**

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also significant predisposing factors for the development of neonatal sepsis.[5]

Blood culture is the primary method for diagnosing neonatal sepsis, but its effectiveness can be limited by factors such as low positivity rates, influenced by factors like volume of the blood, antibiotic usage, and competences of the laboratory. In the emerging economy countries, the culture-negative sepsis is common. Current recommendations suggest a minimum blood volume of 1 ml for cultures, yet most samples received are below 0.5 ml. Increasing the number of blood cultures could improve results, but the process still takes four days.[6] Therefore, there's a pressing need for swift and accurate diagnosis. Biomarkers offer promise, with ideal features including high sensitivity, specificity, bedside monitoring, and cost-effectiveness. Several biomarkers, including PCT, CRP, IL-8, IL-6, IL-10, presepsin, CD64, TNF α, interferon γ, serum amyloid A, and LBP, have been studied for assessing sepsis severity.[7]

C-reactive protein (CRP) serves as a conservative inflammation marker, synthesized in the liver and induced by interleukins. Elevated CRP levels typically appear within 6-8 hours after pathogen exposure, peaking around 36-50 hours later. However, given the critical nature of sepsis, there's a pressing demand for improved and earlier biomarkers. Hofer *et al*. emphasized CRP's role as a humoral marker for bacterial invasion and its importance in monitoring treatment response and guiding antimicrobial therapy. CRP functions as both a diagnostic and prognostic indicator across various conditions.[8]

Inspite of major advances in the treatment and management of sepsis, neonatal sepsis is still one of the foremost reasons of illness and deaths in the intensive care units of neonates.[9] The diagnosis of the neonatal sepsis was made by history, clinical findings such as hyperthermia, hypothermia, decreased blood pressure, deprived blood supply with developing pallor, increased or decreased heart rate, intercostal retraction, cessation of breathing, distress in respiration, grumbling, decreased entry of oxygen, prickliness, lassitude, hypotonia, fits, feed intolerance and distension of the abdomen.[10, 11]

Taking account the scenario in our country, when equal and state of art health services are not available to each and every mother, its need of the hour to discover new horizons of noninvasive biomarkers that facilitate neonatal sepsis diagnosis at the earliest and quickest possible time [(12)]. The present study aimed to determine the beneficial use of hematological markers and serum CRP in premature diagnosis of neonatal sepsis taking blood culture as a benchmark that help to receive results in much shorter time than gold standard (blood culture). To determine the accuracy of TLC, Neutrophil count and CRP protein for rapid diagnosis of Neonatal sepsis. 5

MATERIAL AND METHODS

The present study is approved by the Institutional Ethical Review Committee vide Ref no: 418/2022 dated 6th December 2022. It was a cross-sectional study, conducted in the Department of Microbiology, Combined Military Hospital, Lahore, performed from April 2023 to December 2023. Non-probability purposive sample technique was used for data collection**.** The sample size was calculated by the WHO sample size formula, by taking 92.5% specificity and 77.6% sensitivity with the calculated sample size of 241 [(13)]. Neonates (up to 28 days) with neonatal sepsis were included in the inclusion criteria while exclusion criteria include neonates with anomaly, birth asphyxia, meconium aspiration, expulsions or birth at gestational age < 37 weeks was excluded from the study.

Blood was drawn from the peripheral vein afterward skin antisepsis with an alcohol antiseptic. About 0.5-1 mL of blood from peripheral vein for blood culture. Bottles of the blood cultures was incubated for at 37ºC in Bactec/Alert 3D. Direct Gram staining was be done. Blood and MacConkey agar plates were inoculated and reported the next day for any growth. Smear was made and Gram stained for any growth and detected microorganisms were further identified using 10S, 20S and Vitek2 and tested for antimicrobial sensitivity testing conferring to CLSI 2024.

The data was analyzed by Statistical Package for the Social Sciences (SPSS) version 24. The sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) and Diagnostic accuracy (DA) were calculated by 2×2 model. The p value of ≤ 0.05 was significant while p value of ≤ 0.001 was highly significant.

RESULTS

Out of total 241 neonates, there were male 150 (62%) to female 91(38%) with ratio of 1.6:1. The mean age of the neonates was 9.00±10.72 days. A total of 49 (20.3%) blood culture were positive and proved of neonatal sepsis, out of 241 in total. Whereas 192 (79.6%) were not yielded any growth. Among the isolated microorganisms, Among the isolated microorganisms, *Acinetobacter baumannii* 8(17%), *Acinetobacter jhonsonii* 3(6%), *coagulase negative Staphylococci* 15(31%), *Methicillin resistant Staphylococci* 3(6%), *Enterococcus faecalis* 3(6%), *Klebsiella pneumoniae* 8(16%), *Listeria monocytogenes* 1(2%), *Pseudomonas aeruginosa* 2(4%), and *Serratia marcescens* 6(12%), as shown in figure-I.

The effect of different variables on the sensitivity, specificity, PPV, NPV and Diagnostic accuracy was calculated by taking Blood culture as gold standard. The result shown in the table-I.

In the present study, antimicrobial sensitivity testing performed against the antimicrobial drugs recommended by CLSI 2024. Antimicrobial resistance pattern of the isolated gram-positive microorganisms shown in Table-II. Antimicrobial resistance pattern of the isolated gram-negative microorganisms shown in Table-III.

Table-I: Effects of different variables on diagnosis of neonatal sepsis (n=241).

Variables	Blood culture		Total	Sensitivity %	Specificity %	PPV %	NPV %	DA %	p-value
	Positive	Negative							
Stratification by age									
Age < 15	37	140	177	75.5	72.9	20.91	81.32	36.93	0.714
Age > 15	12	52	64						
Stratification by Gender									
Male	16	75	91	32.6	39.1	17.52	78.03	55.23	0.409
Female	33	117	150						
Stratification by CRP levels									
Raised	39	7	46	79.6	96.4	84.81	94.82	92.94	≤0.001
Not Raised	10	185	195						
Stratification by TLC value									
Raised	9	6	15	18.5	33.3	60.02	93.03	20.71	≤0.05
Not raised	40	3	43						
Stratification by Neutrophil count									
Raised	15	4	19	30.6	55.5	78.92	12.83	34.54	0.416
Not Raised	34	5	39						

Table-II: Antimicrobial resistance pattern of the isolated gram-positive microorganisms n(%), (n=22).

Antimicrobial drugs	*MRSA* (15) n (%)	*CONS* (3) n (%)	*Enterococci* (3) n (%)	*Listeria monocytogenes* (1) n (%)
Penicillin	15(100)	15(100)	0	0
Erythromycin	15(100)	10(66.66)	-	-
Clindamycin	10(66.66)	5(33.33)	-	-
Doxycycline	5(33.33)	10(66.66)	-	-
Co-trimoxazoles	10(66.66)	5(33.33)	1(33.33)	0
Cefoxitin	15(100)	0	-	-
Vancomycin	0	0	0	-
Linezolid	0	0	0	-

Table-III: Antimicrobial resistance pattern of the isolated gram-negative microorganisms n (%), (n=27).

Antimicrobial drugs	*Acinetobacter baumannii* (8) n(%)	*Acinetobacter jhonsanii* (3) n(%)	*Klebsiella pneumoniae* (8), n(%)	*Serratia marcescens* (6), n(%)	*Pseudomonas aerouginosa* (2), n(%)
Ampicillin	-	-	-	-	-
Ceftriaxone	-	-			-
Coamoxiclav	-	-	6(75.00)	-	-
Pippracillin tazobactam	5(62.50)	0	2(25.00)	3(50.00)	0
Ceftazidime	6(75.00)	0	5(62.50)	2(33.33)	1(50.00)
Cefipime	3(37.50)	0	3(37.50)	2(33.33)	1(50.00)
Imipenem	4(50.00)	0	3(37.50)	1(16.66)	0

Meropenem	2(25.00)	0	1(12,25)	0	0
Doxycycline	0	0	6(75.00)	2(33.33)	-
Gentamicin	4(50.00)	0	6(75.00)	2(33.33)	1(50.00)
Amikacin	5(62.50)	0	6(75.00)	1(16.66)	1(50.00)
Cotrimaxazole	3(37.50)	0	5(62.50)	3(50.00)	-

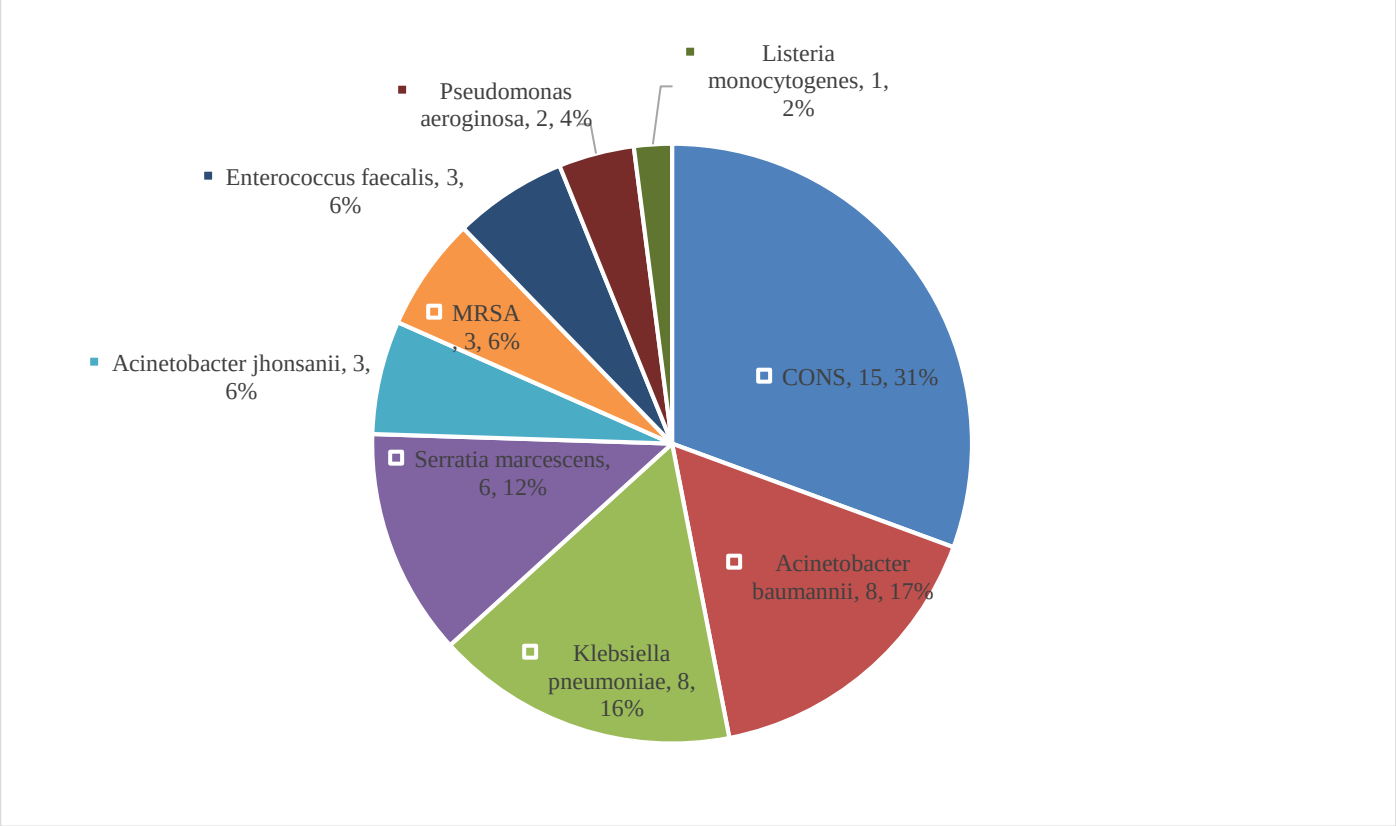

Figure-I: Frequency distribution of various isolates from neonatal sepsis.

DISCUSSION

The incidence of the neonatal sepsis is on the rise. The male 150 (62%) to female 91(38%) ratio is 1.6:1 which is also in consistent to the study performed earlier in which 58% of males and 42% of the females are observed [(14)]. The mean age of the neonate was 9 days which is in accordance to a study performed earlier.[15]

Coagulase negative Staphylococci 15(30.6%), is the most widely isolated microorganism followed by *Acinetobacter baumannii* 8(16.3%), *Klebsiella pneumoniae* 8(16.3%), *Serratia marcescens* 6(12.2%), *Acinetobacter jhonsonii* 3(6.1%), *Methicillin resistant Staphylococci* 3(6.1%), *Enterococcus faecalis* 3(6.1%), 2(4.1%) *Pseudomonas aeruginosa*, and *Listeria monocytogenes* 1(2.0%). In a study conducted already in Lahore, Pakistan also demonstrated CONS as most commonly isolated microorganism in the neonatal sepsis which is in consistent to the finding of present study.[16] In another study, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus were isolated which is also in consistent with the present study.[17] In the present study, Listeria monocytogenes was recovered from a case of Early-onset neonatal sepsis which is in consistent with the finding of the previous study.[18]

In the present study, majority of the Gram-positive bacterial strains not sensitive to penicillin and most sensitive to linezolid and vancomycin. the same findings were observed in the study performed earlier in which no gram-positive isolate was resistant to linezolid and vancomycin.[19] The gram-negative bacteria are most resistant to ampicillin and the least resistant is noted against carbapenems. Same findings were observed in the study performed previously.[20]

In a study performed by Worku with his colleagues in 2022, found out the beneficial role of total leukocyte count in the early diagnosis of neonatal sepsis as compared with the blood culture that may delay the

diagnosis leading to clinical morbidity.[21] In another study performed by Agnello *et al*., in 2021 signify complete blood count as a precious test for the early diagnosis of the sepsis that may lead to early diagnosis of the sepsis and leads to increased clinical efficacy. Moreover, the author regarded the complete blood examination as a cheap, easy to perform and readily available test as compared to the blood culture for the rapid diagnosis of the neonatal sepsis.[22]

In a study performed by Shoukry *et al* in the 2021, the author highlighted the value of CRP protein for the early diagnosis of the neonatal sepsis. Furthermore, the author regarded CRP protein as a new diagnostic strategy for the rapid diagnosis of the neonatal sepsis as compared to the non-septic neonates.[23] In another study performed by Jalali *et al*., in 2024, phrased the importance of the complete blood examination and CRP protein as a readily available and easy to performed and non-expensive test for the diagnosis of the neonatal sepsis. [24] The findings of the above-mentioned studies are in consistent to the finding of the present study in which the value of complete blood count, TLC, DLC and CRP protein proved to be a useful indicator for the early diagnosis of the neonatal sepsis.

CONCLUSION

The present study helps to identify the role of hematological indicators as TLC, neutrophil count and CRP, as rapid indicator of neonatal sepsis. The results of the hematological markers and serum CRP received in a much less time as compared to the blood culture that gives results after the delay of 4 days. This will reduce the burden of overuse and misuse of antibiotics in the culture negative neonates that leads to damaged and resistant flora with short- & long-term hazardous complication and consequence because antibiotic treatment is initiated to most of the neonates with suspicion of sepsis, but most of the culture turn to be negative.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Zill-E-Huma: Main conception of the study, study design, manuscript writing, data collection, data analysis, final approval, accountable for every aspect of this research work

Muhammad Abid Farooque: Study design, data collection, data analysis, final approval, accountable for every aspect of this research work

Muhammad Yasir Rafiq: Study design, data analysis, final approval, accountable for every aspect of this research work

Chahat Hussain, Asif Younis and Syed Muhammad Faizan: Data collection, data analysis, final approval, accountable for every aspect of this research work

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