Cross-sectional Study

Diagnostic utility of combined immature and total neutrophil counts along with C-reactive protein in early detection of neonatal sepsis: A cross-sectional study

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ABSTRACT

Introduction: A timely diagnosis is critical for management of Neonatal sepsis. Blood Culture is considered to be the "Gold Standard" for its diagnosis, but it has some limitations. In recent times, highly sensitive and specific inflammatory markers like interleukins, ELISA, counter immune-electrophoresis etc. have been in use for its diagnosis. But these are impractical for developing countries, due to their high cost and requirement of sophisticated equipments. A combination of haematological parameters like total leucocyte count (TLC), immature to total neutrophil ratio (I/T ratio), absolute neutrophil count (ANC), platelet count and C-reactive protein (CRP) estimation provide an early diagnosis of bacteremia. This study was undertaken to evaluate the usefulness of the above mentioned parameters as indicators for early diagnosis of neonatal sepsis.
Material and methods: In the present cross-sectional study, we intent to analyse various hematologic parameters in 160 neonates admitted in the neonatal care unit of a tertiary care hospital in Delhi. We obtained data from the records of blood culture and complete blood counts of neonates from pathology and microbiology departments of the hospital. Out of 160 admitted neonates, 80 were taken as cases and remaining 80 were taken as controls. Medical records were studied to identify infants born at ≥ 34 weeks gestation. CBCs was analysed, blood cultures and CRP were done in department of Microbiology. CBC, CRP and Blood culture was done as per standard protocols and clinical assesment by paediatrician. The statistical analyses were performed using SPSS version 22 for windows.
Results: Among 80 neonates, who were in early neonatal sepsis, 44 cases (55%) were females, and 36 (45%) were males. The Microbiological profile of 80 septic neonates was analysed. The I/T value, ANC and CRP values were significantly higher in the neonates suffering from sepsis as compared to the control group. Among 80 septic neonates (cases), 30 (37.5%) were having normal ANC while 50 (62.5%) were having increased ANC and 34 (42.5%) were having normal I:T ratio while 46 (57.5%) were having increased I:T ratio. Out of 80 septic neonates (cases), 18 (22.5%) were having normal CRP while 62 (77.5%) were having increased CRP.
Conclusion: ANC, I/T Ratio and CRP are quick, simple and cost-effective routine laboratory tests which help in early diagnosis of neonatal sepsis. Although there are many serological markers available, ANC and I/T Ratio serves as a reliable predictor of neonatal sepsis. With a good sensitivity, high specificity and a good negative predictive value these parameters can therefore help in timely and early identification of neonatal sepsis.

1. Introduction

According to World Health Organization (WHO), perinatal deaths are responsible for maximum cases of the childhood mortality in children aged below 5 years especially in developing countries like India [1]. Neonatal infections are the most common cause of perinatal mortality [2,3]. In India according to National Neonatal Perinatal Database (NNPD, 2020), the incidence of neonatal sepsis is 18 per 1000 live births [4]. Neonatal sepsis is a clinical syndrome characterized by classical signs and symptoms associated with bacteraemia [5]. Initial warning

signs and symptoms of sepsis are mostly non-specific and have different presentation in various gestational ages making it difficult in establishing an early clinical diagnosis. Making a timely diagnosis therefore is critical for early diagnosis [6,7].

In Europe and North America, Group B Streptococcal disease is the leading cause of neonatal sepsis, but in tropical and developing countries gram negative organisms predominates in majority of cases [8]. According to NNDP data, in India the disease is most frequently caused by *Klebsiella pneumoniae* followed by *Staphylococcus aureus* [9]. Although Blood Culture is considered as the "Gold Standard" for its diagnosis, but it has some associated limitations like it is time consuming, has low positivity and false positive results due to sample contamination [10]. In recent times, highly sensitive and specific inflammatory markers like interleukins, ELISA, haptoglobins, counter immune-electrophoresis etc. have been in use for its diagnosis [11]. But these are impractical for developing countries like India, due to their high cost and requirement of sophisticated equipments. Cheap, easily performed, quick and reliable tests like complete blood count (CBC) with different neutrophil parameters and C-reactive protein (CRP) are frequently used [12]. A combination of haematological parameters like total leucocyte count (TLC), immature to total neutrophil ratio (I/T ratio), absolute neutrophil count (ANC), platelet count and C-reactive protein (CRP) estimation provide an early diagnosis of bacteremia [11,12]. These bed side tests are cost-effective and can be performed within a short time before the start of empirical antibiotic therapy in neonate. This helps in avoiding the overtreatment and development of antibiotic resistance thus reducing burden of high cost in underprivileged settings [13]. This study was undertaken to evaluate the usefulness of the above mentioned parameters as indicators for early diagnosis of neonatal sepsis.

2. Material and methods

The present Cross-sectional study was conducted in Departments of Pediatrics, Pathology and Microbiology, North Delhi Medical College and Hindu Rao Hospital Delhi, over a period of one year (2020-21) on 160 neonates admitted in the neonatal care unit of our Hospital. A total of 160 cases were included in the study after obtaining the Ethical clearance from the Institutional Review Board, vide no. Dean/North DMC/MC/2021/1030 Dated 27-12-2021(Certificate attached).

In the present study, we intent to analyse various hematologic parameters in 160 neonates admitted in the neonatal care unit of a tertiary care hospital in Delhi. Infants with predisposing perinatal factors or with clinical suspicion of sepsis were included. The study included two groups: Group 1—Infants with sepsis with positive blood cultures and Group 2—Normal infants with negative blood culture.

We obtained data from the records of blood culture and complete blood counts of new born from pathology and microbiology departments of the hospital. Out of 160 admitted neonates, 80 were taken as cases and remaining 80 were taken as controls. Medical records were studied to identify infants born at $\geq$ 34 weeks gestation. Only those infants were included who had a CBC done at <72 h of age and within 1 h of a blood culture. CBCs was analysed using Sysmex haematology analysers in haematology laboratory. Blood cultures and CRP were done in department of Microbiology by Bactec method and automated analyser respectively.

CBC, CRP and Blood culture was done as per standard protocols and clinical assesment by paediatrician. The differential WBC counts and peripheral blood examination was done manually for identification of band forms. The ANC was calculated as the automated estimate of the WBC $\times$ (% segmented neutrophils + % bands)/100. I/T ratio were calculated as the total number of immature neutrophils (promyelocytes, myelocytes, metamyelocytes and bands) divided by the total number of cells in the neutrophilic cell line (immature plus segmented neutrophils). The work has been reported in line with the STROCSS criteria [14].

2.1. Statistical analysis

Sensitivity, specificity and positive and negative predictive values were evaluated using standard statistical methods. $P < 0.05$ was considered as significant statistical difference. Comparison was made using Chi square test. The statistical analyses were performed using SPSS version 22 for windows.

3. Results

Among 80 neonates, who were in early neonatal sepsis, 44 cases (55%) were females, and 36 (45%) were males. In the non-septic group, 50 neonates (62.5%) were females and 30 (37.5%) were males.

Table 1 shows Microbiological profile of 80 septic neonates, 24 cases (30%) were coagulase negative staphylococcus, 04 (5%) were klebsheilla pneumoniae, 34 (42.5%) were staphylococcus aureus, 10 (12.5%) were *Pseudomonas aeruginosa* and 8 (10%) were Enterococcus (Fig. 1).

According to the findings, I/T value, ANC and CRP values were significantly higher in the septic neonates compared to the control group. Among 80 septic neonates (cases), 30 (37.5%) were having normal ANC while 50 (62.5%) were having increased ANC. In the non-septic group (control), 72 neonates (90%) were normal ANC and 08 (10%) were having high ANC (Table 2).

Among 80 septic neonates (cases), 34 (42.5%) were having normal I: T ratio while 46 (57.5%) were having increased I:T ratio. In the non-septic group (control), 70 neonates (87.5%) were normal and 10 (12.5%) were having high I:T ratio (Table 3).

Out of 80 septic neonates (cases), 18 (22.5%) were having normal CRP while 62 (77.5%) were having increased CRP. In the non-septic group (control), 28 neonates (35%) were having normal CRP and 52 (65%) were having high CRP (Table 4).

Sensitivity, Specificity, and Negative and Positive Predictive Values of Evaluated Parameters (%) in Sepsis Proven Population (n = 80) is been highlighted in Table 5.

4. Discussion

The high mortality and morbidity associated with neonatal sepsis prompts for an early diagnosis which is very crucial for the management of these neonates. High index of clinical suspicion is also required as presenting clinical manifestations vary and are non-specific. No single laboratory test independently highlights neonatal sepsis and so a combination of laboratory tests helps in predicting neonatal sepsis with certainty [15,16].

Over the years, because of its simple, cost-effective method, the significance of HSS score in predicting neonatal sepsis has been validated. Neonatal sepsis remains as a potentially life-threatening disease especially in developing countries like India. Risk factors include maternal factors like maternal infections, premature rupture of membranes, various procedures etc. and risk factors in infants include, poor cord care, low birth weight, various congenital anomalies, low APGAR score [17]. Patients may present with complaints of respiratory distress, hypothermia, irritability, hypo or hyperglycaemia, vomiting, poor feeding, seizures and shock [18]. It remains a great challenge to diagnose neonatal septicaemia as the early signs of sepsis [19].

Table 1
Bacteriological profile in the blood culture positive cases (n = 80).

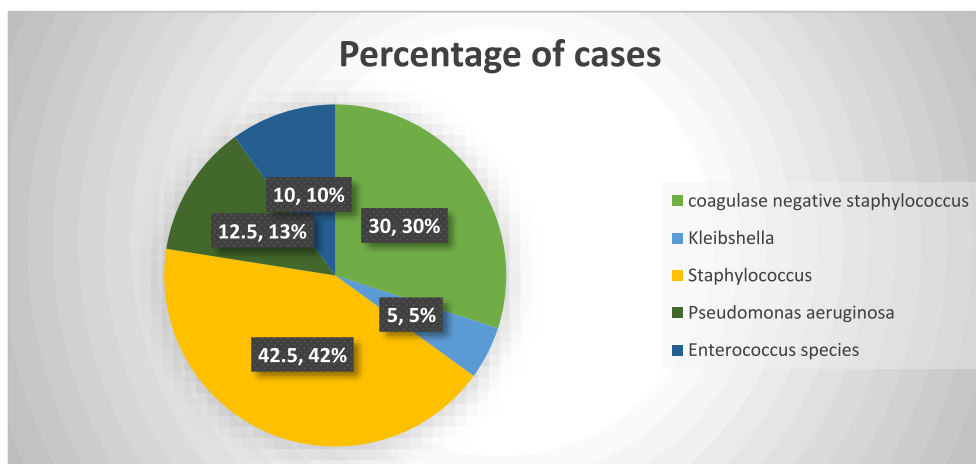| S No.	Microbiological profile (organism)	No. of cases (n = 80)
1	Coagulase negative Staphylococcus	24 (30%)
2	Klebsiella pneumonia	04 (5%)
3	*Staphylococcus aureus*	34 (42.5%)
4	*Pseudomonas aeruginosa*	10 (12.5%)
5	Enterococcus species	08 (10%)

Fig. 1. Showing distribution of cases as per bacteriological profiles in the Blood Culture.

Table 2
Absolute neutrophil counts in the two study groups.

ANC	Control (n = 80)Sepsis probable	Cases (n = 80)Sepsis proven
Normal	72 (90%)	28 (35%)
Increased	08 (10%)	46 (57.5%)
Decreased	00 (0%)	06 (7.5%)

Table 3
Immature to total neutrophil count ratio in the two study groups.

I:T ratio	Control (n = 80)Sepsis probable	Cases (n = 80)Sepsis proven
Normal	70 (87.5%)	34 (42.5%)
Increased	10 (12.5%)	46 (57.5%)

Table 4
C-reactive protein values in the two study groups.

CRP	Control (n = 40)Sepsis probable	Cases (n = 40)Sepsis proven
Normal	28 (35%)	18 (22.5%)
Increased	52 (65%)	62 (77.5%)

Table 5
Comparative analysis of tests used in proven sepsis population (n = 80).

Parameter	ANC	I/T	CRP	COMBINED
Sensitivity (%)	90.00	87.50	77.5	98.7
Specificity (%)	62.50	57.50	65.0	83.66
PPV (%)	70.59	67.31	54.4	68.3
NPV (%)	86.21	82.14	60.9	95.2
Accuracy (%)	76.25	72.50	75.3	99.3

Screening tests evaluation for neonatal sepsis is an absolute necessity to prevent a serious threat to the baby. Neonates which are noninfected should also be identified so as to avoid antibiotic administration and to prevent the emergence of resistant microorganisms [20]. Ideally a screening test must have high sensitivity and high negative predictive values. Risk of missing a sepsis prone patient with a certain infection is higher than the risk of antibiotics over treatment, so a low specificity and low positive predictive value are acceptable [21]. Although the gold standard test for diagnosing sepsis is blood culture, but the procedure is time consuming and expensive as it takes 48–72 h [22]. Also the technique requires a well-equipped laboratory setup which is mostly non available in most of the community hospitals [23].

In our study, individual parameters like Absolute neutrophil count and I:T ratio showed high sensitivity and negative predictive value in prediction of neonatal sepsis which was in consistence with many other studies [24–27].

4.1. Limitation of the study

Limitations of this study were that correlation with laboratory data like C-reactive protein, micro ESR etc., could not be done due to some technical difficulties, which otherwise if done would have increased the validity of the study.

5. Conclusion

ANC, I/T Ratio and CRP are quick, simple and cost-effective routine laboratory tests which help in neonatal sepsis prediction. Although there are many serological markers available, ANC and I/T Ratio serves as a reliable predictor of neonatal sepsis. With a good sensitivity, rather than high specificity and a good negative predictive value these parameters can therefore help in timely and early identification of neonatal sepsis.

Ethical approval

Approved By Iec Via Letter No.
Dean/North DMC/MC/2021/1030 DATED:27/12/2021.

Sources of funding for your research

NIL.

Author contributions

Dr. Namita Bhutani: HAVE WRITTEN THE MANUSCRIPT.
Dr. Sumit Jethani: SUPERVISION OF THE STUDY.
Dr. Abhishek Yadav: DATA COLLECTION.

Registration of research studies

Approved by IEC via letter no. Dean/North DMC/MC/2021/1030 DATED:27/12/2021.

Consent

Written informed consent was obtained from the parents of the patients for publication of this study and accompanying images. A copy of the written consent is available for review by the editor–in–chief of this journal on request.

Guarantor

DR. NAMITA BHUTANI.

Data availability statement

Data will be available to all the readers as per journal's rules.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Declaration of competing interest

NIL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2022.103589.

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