



A STUDY ON HEMATOLOGICAL PARAMETERS, COAGULATION PROFILE AND C-REACTIVE PROTEIN IN NEONATAL SEPSIS IN TERTIARY CARE HOSPITAL

Pathology

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ABSTRACT

Neonatal sepsis is defined, as a disseminated disease with positive blood culture during the first month of life.

To find out utility of peripheral blood smear in neonatal sepsis by evaluating total leucocyte count, platelet count, toxic granules, immature to total neutrophil ratio. Descriptive observational study was done Department of Pathology (hematology division) and sick neonatal care unit (SNCU) under Department of Pediatric medicine of RGKMCH for 18 month. 84 neonates diagnosed as sepsis and suspected as sepsis on the basis of clinical signs and symptoms. Late onset neonatal sepsis was more common than early onset neonatal sepsis depending on the duration. In this study mean age of affected neonates were 5.53 days. so most of the neonates affected in the first week of life. In the present study 78.6% of cases were showing positive growth in blood culture. Blood culture is considered as gold standard for diagnosis of neonatal sepsis. so in the present study, the different hematological parameters, coagulation profile and C- reactive protein were compared with blood culture results, as the blood culture were considered as gold standard for diagnosis of neonatal sepsis. Low birth weight neonates were more commonly affected with neonatal sepsis compared to the neonates who were more than 2.5kg. We found that CRP Sensitivity was 97.0, Specificity: 72.2, Positive Predictive Value: 92.8 and Negative Predictive Value: 86.7 It was found that platelet Sensitivity was 72.7, Specificity: 77.8, Positive Predictive Value: 92.3, Negative Predictive Value: 43. Association of platelet vs. blood culture was statistically significant ($p=0.00009$). It was found that ANC Sensitivity was 71.2, Specificity: 72.2, Positive Predictive Value: 90.4, Negative Predictive Value: 40.6.

KEYWORDS

Hematological Parameters, Blood culture, Coagulation Profile, C- Reactive Protein, Neonatal Sepsis

INTRODUCTION

Neonatal sepsis is defined, as a disseminated disease with positive blood culture during the first month of life. It is one of the important cause for mortality and morbidity in neonates especially in developing countries. Mortality rate in neonatal sepsis differs according to the type of organism involved, most common causes of neonatal sepsis are group B streptococci (GBS), Escherichia coli (E.COLI) and Listeria monocytogenes in developed countries where as gram negative bacteria and coagulase negative staphylococci (CONS) in developing countries.¹

High cost of antibiotics overburdens the already underprivileged parents. Blood culture is still considered to be the gold standard, however its accuracy has been questioned because of spurious positive results due to contamination and negative blood culture in fatal generalized bacterial infections, yield of a positive blood culture ranges from 8-73% as shown in various studies. Moreover, the technique of blood culture is time consuming and demands a well-equipped laboratory, which is not available in many of the community hospitals.²

Therefore, there is need for a test that is cheap, easily performed with quick availability of reports. ideal diagnostic test for neonatal sepsis should have maximum sensitivity and specificity. In recent years, various investigators have evaluated some inflammatory markers (e.g. Procalcitonin, haptoglobins, interleukins, etc) to diagnose neonatal sepsis. Although these markers are sensitive and specific, they are sophisticated and expensive and hence, not suitable for developing countries. Various cheap and reliable laboratory tests have been evaluated for the diagnosis of systemic infection in neonates. Complete blood count with the various parameters and c-reactive protein are most frequently used.

1. To find out utility of peripheral blood smear in neonatal sepsis by evaluating total leucocyte count, platelet count, toxic granules, immature to total neutrophil ratio.
2. To find out relationship between hematological parameters and coagulation profile in neonatal sepsis.

MATERIALS AND METHODS

Study design- Descriptive observational study was done Department of Pathology (hematology division) and sick neonatal care unit (SNCU) under Department of Pediatric medicine of RGKMCH for 18 month. 84 neonates diagnosed as sepsis and suspected as sepsis on the basis of clinical signs and symptoms.

Inclusion criteria – All neonates diagnosed as sepsis or suspected as sepsis on the basis of clinical sign and symptoms.

Exclusion criteria – Neonates who had already received or receiving antibiotics. All congenital malformations and metabolic diseases were excluded from study.

In this study all the neonates with sign and symptoms of sepsis were admitted to SNCU. Those who already received antibiotics were not included in the study. For sepsis screen 4 ml blood of venous blood was drawn using a sterile plastic syringe with 23 G needle with proper aseptic precautions. Out of this 4ml of blood sample 1 ml was inoculated in the blood culture bottle and further, another 1 ml blood was inoculated into the clotted vial for estimation of C- reactive protein. The remaining 2ml of blood was collected in the vacutainer containing EDTA as anticoagulant was used for estimation of hematological parameters through cell counter and also for preparation of peripheral blood smear. For coagulation profile 1 ml of venous blood samples were collected with sterile packed plastic syringe along with 23G needle. After capping the vacutainer the blood and anticoagulant were mixed immediately by gentle inversion of 5 times. The anticoagulant and blood ratio were very important for coagulation profile study. Ratio should be in such a way that anticoagulant : blood proportion being 1:9. Coagulation studies. Blood samples were examined within 2 hours of collection. In this study 0.9ml venous blood and 0.1ml trisodium citrate ratio was maintained strictly. All the procedure were done with the consent of parents or legal guardians of neonates. All procedures were done with proper aseptic precaution along with proper waste management at right place. Statistical analysis: In this study statistical analysis was done by SPSS 27 software (SPSS Inc., Chicago, IL, version 16 for windows).

RESULT AND ANALYSIS

We found that 28(33.3%) patients had ≤ 3 days of age and 56(66.7%) patients had > 3 days of age. Our study showed 39(46.4%) patients had female and 45(53.6%) patients had male. It was found that 66(78.6%) patients had positive blood culture. We showed that 39(46.4%) patients had normal P-time.

We found that 34(40.5%) patients had normal INR. Our study showed 29(34.5%) patients had normal APTT. It was found that 3(3.6%) patients had normal CRP. We showed that 78(92.9%) patients had normal TLC. We found that 22(26.2%) patients had normal platelet.

Our study showed 27(32.1%) patients had normal ANC. It was found

that 34(40.5%) patients had I/T ratio <0.2 and 50(59.5%) patients had I/T ratio >0.2 . We showed that 11(13.1%) patients had band cell, 12(14.3%) patients had degenerated cells, 33(39.3%) patients had thrombocytopenia and 28(33.3%) patients had toxic granules.

We found that 17(20.0%) patients had other cause, 55(65.5%) patients had prematurity and 12(14.3%) patients had PROM. Our study showed that mean age (mean $\pm$ s.d.) of patients were 5.5357 ± 3.9983 days. It was found that Association of age in days vs. blood culture was not statistically significant ($p=0.3974$).

Our study showed was found that positive blood culture, 31(47.0%) patients had female sex and 35(53.0%) patients had male sex. Association of sex vs. blood culture was not statistically significant ($p=0.9392$). In positive blood culture, 37(56.1%) patients had weight <2.5 and 29(43.9%) patients had weight ≥ 2.5 . Association of weight vs. blood culture was not statistically significant ($p=0.0872$).

We showed that Association of P time vs. blood culture was not statistically significant ($p=0.9392$). We found that Association of INR vs. blood culture was not statistically significant ($p=0.9075$). In Our study showed positive blood culture, 43(65.2%) patients had abnormal APTT and 23(34.8%) patients had normal APTT. Association of APTT vs. blood culture was statistically significant ($p=0.0045$).

We showed that 64(97.0%) patients had abnormal CRP and 2(3.0%) patients had normal CRP. It was found that Association of CRP vs. blood culture was statistically significant ($p<0.0001$). We showed that Association of TLC vs. blood culture was not statistically significant ($p=0.8248$).

In Our study showed positive blood culture, 48(72.7%) patients had abnormal platelet and 18(27.3%) patients had normal platelet. Association of platelet vs. blood culture was statistically significant ($p=0.00009$). We showed that in positive blood culture, 47(71.2%) patients had abnormal ANC and 19(28.8%) patients had normal ANC. Association of ANC vs. blood culture was statistically significant ($p=0.0007$).

Our study showed Association of I/T ratio vs. blood culture was not statistically significant ($p=0.9075$). We showed that Association of peripheral smear vs. blood culture was not statistically significant ($p=0.9129$). It was found that Association of maternal history vs. blood culture was not statistically significant ($p=0.0500$).

DISCUSSION

In this study association of sex vs. blood culture was not statistically significant ($p=0.9392$).

Khair KB et al 3 (2010) explained, 101 neonates with clinical features of neonatal sepsis were included in the study. Out of all the cases, 59 (58.4%) cases were ≤ 7 days of age. Early onset sepsis was present in 58.4% of cases and late onset sepsis was present in 41.6% of the cases. Bacterial culture was positive in 27 (26.7%) cases. Chandra A et al (1988) 4 explained, males were more affected compared to females in sepsis. Buch et al. (2011) 5 the incidence of neonatal sepsis was higher in male (60%) than female neonates. So, on comparing those above four studies, it was found that the present study showed similarity, considering the age of the patients and the mean age of patients were 5.53 days who were affected with sepsis. Panwar C et al 6 (2017) found, Among the various hematological parameters like toxic granules, raised Micro-ESR, I/T Ratio >0.2 , thrombocytopenia remained important markers for early diagnosis of culture positive EOS ($p<0.05$).

Duhan A et al 7 (2016) found that, I: T ratio, total PMN count, I:M ratio and Immature PMN count were found to have high sensitivity while degenerative changes in PMN was found to be highly specific test for sepsis along with the total WBC count. Ghosh et al (2001) 9 and Manisha Makkar et al (2013) 10 Elevated I/T ratio was found to be quite reliable hematological indicator of neonatal sepsis with sensitivity 63.4%, specificity 92% and PPV 96.3%. They showed that immature PMN count and I:T PMN ratio was also a very sensitive indicator of neonatal sepsis.

Basu S et al (1999) 11 showed that thrombocytopenia is associated with poor prognosis. The above seven studies had shown the statistical significance of I/T ratio, low platelet count, band cells in diagnosis of

neonatal sepsis. In the present study the peripheral smear findings were concordant with the above seven studies, but the findings were not statistically significant in the present study. In this study it was found that 55(65.5%) patients had history of prematurity and 12(14.3%) patients had history PROM. 17(20.0%) patients had history of other maternal cause. In positive blood culture, 37(56.1%) patients had weight <2.5 and 29(43.9%) patients had weight ≥ 2.5 . Association of weight vs. blood culture was not statistically significant ($p=0.0872$).

Waseem R (2005) 12 observed septicemia more common in low birth weight and preterm babies. Higher incidence of sepsis in low birth weight, both Preterm and small for gestational (SGA) is because they have low maternal acquired IG and inherent susceptibility to infection. Panwar C et al 13 (2017) explained EOS was seen commonly in preterm.

Shah BA et al 14 (2014) found that neonatal sepsis continues to be a common and significant health care burden, especially in very-low-birth-weight infants (VLBW <1500 g).

Ali SW. et al 15 (2010) Over the study period, 196 patients were evaluated for sepsis on the basis of clinical features. Blood cultures were positive in 76 babies. Thirty six babies had meningitis and 22 babies had pneumonia. Thrombocytopenia, leucopenia, neutropenia, coagulopathy and hyponatremia had a statistically significant relationship with mortality. In the present study, among positive blood culture cases, 43(65.2%) patients had abnormal APTT and 23(34.8%) patients had normal APTT. Association of APTT vs. blood culture was statistically significant ($p=0.0045$). It was found that APTT Sensitivity was 65.2, Specificity: 72.2, Positive Predictive Value: 89.6, Negative Predictive Value: 36. It was found that association of P time vs. blood culture was not statistically significant ($p=0.9392$). Association of INR vs. blood culture was not statistically significant ($p=0.9075$). It was found that platelet Sensitivity was 72.7, Specificity: 77.8, Positive Predictive Value: 92.3, Negative Predictive Value: 43. In positive blood culture, 48(72.7%) patients had abnormal platelet and 18(27.3%) patients had normal platelet. Association of platelet vs. blood culture was statistically significant ($p=0.00009$).

Onyedibe KI et al. (2012) 16 showed that Septicemic cases with bleeding probably suffered more consumption coagulopathy leading to a state of DIC.

Ahmed Z et al 17(2005) found that 67 (81.7%) were showing positive CRP while 58 (70.73%) were showing positive blood cultures. The sensitivity, specificity, positive predictive and negative predictive values and diagnostic accuracy of CRP were 81.7%, 88.0%, 95.7%, 59.5% and 83.2% respectively. It is a good diagnostic test and can give idea about the infection in neonates at the time of initial assessment.

Mehrotra G. et al 18 (2017) found that 34 (68%) had positive CRP while 31 (62%) had positive blood culture. The sensitivity, specificity, positive and negative predictive values of CRP were 90.32%, 42.10%, 71.79% and 72.72% respectively. The qualitative method of estimating CRP which is cheap and rapid has moderate sensitivity, specificity and negative predictive value. In positive blood culture, 64(97.0%) patients had abnormal CRP and 2(3.0%) patients had normal CRP. Association of CRP vs. blood culture was statistically significant ($p<0.0001$). We found that CRP Sensitivity was 97.0, Specificity: 72.2, Positive Predictive Value: 92.8 and Negative Predictive Value: 86.7.

Hofer N et al 19 (2012) found that CRP is useful for monitoring the response to treatment and guiding antibiotic therapy, though nothing replaces the clinical impression and the gold standard (i.e. culture results). In spite of the large amount of research done on CRP in neonates.

Fisher Jr CJ et al 20 (2000) found that blood culture was used as gold standard for diagnosis of NNS. Of 50 neonates studied, 34 (68%) had positive CRP while 31 (62%) had positive blood culture. The sensitivity, specificity, positive and negative predictive values of CRP were 90.32%, 42.10%, 71.79% and 72.72% respectively. The qualitative method of estimating CRP which is cheap and rapid has moderate sensitivity, specificity and negative predictive value.

It was found that platelet Sensitivity was 72.7, Specificity: 77.8, Positive Predictive Value: 92.3, Negative Predictive Value: 43. In

positive blood culture, 48(72.7%) patients had abnormal platelet and 18(27.3%) patients had normal platelet. Association of platelet vs. blood culture was statistically significant ($p=0.00009$). It was found that ANC Sensitivity was 71.2, Specificity: 72.2, Positive Predictive Value: 90.4, Negative Predictive Value: 40.6 In this study, in positive blood culture, 47(71.2%) patients had abnormal ANC and 19(28.8%) patients had normal ANC. Association of ANC vs. blood culture was statistically significant ($p=0.0007$). Association of I/T ratio vs. blood culture was not statistically significant ($p=0.9075$). In this study it was found that, association of TLC vs. blood culture was not statistically significant ($p=0.82489$).

Ahmed Z et al 21 (2005) found that ANC was the second most sensitive test having sensitivity of 71.4 % for group-A and 63.9 % for group-B and 88% specificity. The sensitivity of platelet count was 64.3%. A set of investigations including CRP, TLC, ANC are highly sensitive in detection of culture negative cases of neonatal sepsis.

CONCLUSION

After completion of this study, it was found that ; Late onset neonatal sepsis was more common than early onset neonatal sepsis depending on the duration . In this study mean age of affected neonates were 5.53 days. So most of the neonates affected in the first week of life.

Low birth weight neonates were more commonly affected with neonatal sepsis compared to the neonates who were more than 2.5kg. Prematurity was found to be very important risk factor because most of the neonates who were affected with neonatal sepsis were found to be premature. PROM was found to be very high risk factor for neonatal sepsis. Considering the low number of PROM cases , but most were culture positive. Sepsis may complicate to DIC

In positive blood culture, 64(97.0%) patients had abnormal CRP and 2(3.0%) patients had normal CRP. Association of CRP vs. blood culture was statistically significant ($p<0.0001$). We found that CRP Sensitivity was 97.0, Specificity: 72.2, Positive Predictive Value: 92.8 and Negative Predictive Value: 86.7 It was found that platelet Sensitivity was 72.7, Specificity: 77.8, Positive Predictive Value: 92.3, Negative Predictive Value: 34.

In positive blood culture, 48(72.7%) patients had abnormal platelet and 18(27.3%) patients had normal platelet. Association of platelet vs. blood culture was statistically significant ($p=0.00009$). It was found that ANC Sensitivity was 71.2, Specificity: 72.2, Positive Predictive Value: 90.4, Negative Predictive Value: 40.6 It was found that in positive blood culture, 47(71.2%) patients had abnormal ANC and 19(28.8%) patients had normal ANC.

Table: Association of all parameters in BLOOD CULTURE

		BLOOD CULTURE POSATIVE	BLOOD CULTURE NEGATIVE	TOTAL
AGE GROUP IN DAYS	≤3	24	4	28
	>3	42	14	56
SEX	Female	31	8	39
	Male	35	10	45
WEIGHT	<2.5	37	6	43
	≥2.5	29	12	41
P-TIME group	Abnormal	36	9	45
	Normal	30	9	39
INR group	Abnormal	40	10	50
	Normal	26	8	34
APTT group	Abnormal	43	5	48
	Normal	23	13	36
CRP group	Abnormal	64	5	69
	Normal	2	13	15
TLC group	Abnormal	5	1	6
	Normal	61	17	78
PLATELET group	Abnormal	48	4	52
	Normal	18	14	32
ANC group	Abnormal	47	5	52
	Normal	19	13	32

I/T RATIO	<0.2	26	8	34
	>0.2	40	10	50
PERIPHERAL SMEAR	BAND CELL	8	3	11
	DEGENERATED CELLS	9	3	12
	THROMBOCYTOPENIA	26	7	33
	TOXIC GRANULES	23	5	28
MATERNAL HISTORY	OTHER CAUSES	10	7	17
	PREMATURITY	45	10	55
	PROM	11	1	12

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