Evaluation Of Hematological Parameters In Early Onset Neonatal Sepsis

Dr. Kuhu Pal*, Dr. Arnab Kumar Samanta**

*Assistant Professor, Department of Microbiology, **Clinical Tutor, Department of Pediatrics, College of Medicine and JNM Hospital, WBUHS, Kalyani, Nadia, West Bengal, 741235

Abstracts: **Background & Objective** : Diagnosis of early onset neonatal sepsis (EONS) is always a challange to the neonatologists. So, the study evaluate role of hematological parameters in diagnosis of EONS in a teaching hospital of eastern India. **Methods**: Newborn, age less than 72hours, associated with risk factors or clinical diagnosis of sepsis, admitted between March 2011 and February 2013 was enrolled in this study. They were subjected to blood culture and various sepsis screen parameters like C-Reactive Protein, micro-erythrocyte sedimentation rate (Micro ESR), total leukocyte count (TLC), Immature to Total Neutrophil ratio (I:T ratio) and platelet count. Sensitivity, specificity, positive predictive value(PPV), negative predictive value (NPV) as well as P value of all the parameters were calculated considering blood culture as 'gold standard' for sepsis. **Result:** Among 238 clinically suspected cases of early onset neonatal sepsis, blood culture was positive in 90(37.8%) cases of which *Klebsiella pneumoniae* (45.71%) was the predominant organism. Positive CRP and Micro ESR were the most useful tests for sepsis with a high specificity of 91.89% and 90.54% respectively but sensitivity of any one was not satisfactory. Negative predictive value of the CRP, micro- ESR, I:T ratio ranges between 90.07% and 81.99% . In relation to sepsis alteration in hematological parameters were found statistically significant (P value <0.05).**Conclusion:** The value of the hematological tests could be used satisfactorily to rule out EONS and withheld unnecessary antibiotic treatment. Thus the tests aids in management of EONS while waiting for culture report. [Pal Kuhu et al NJIRM 2013; 4(6) : 29-34]

Key Words: C Reactive Protein, EONS, Hematological Parameter.

Author for correspondence: Dr Kuhu Pal, Assistant Professor, Department of Microbiology, College of Medicine and JNM Hospital, WBUHS, Kalyani, Nadia, West Bengal,Pin 741235. e- mail: kuhupal18@gmail.com

Introduction: Clinical diagnosis of sepsis in new born is not easy because of nonspecific symptoms and signs. But early recognition, diagnosis and treatment of serious infection in the neonate is essential to confer a healthy life to those newcomers to the world. Blood culture is the gold standard for the diagnosis of the neonatal sepsis but it usually takes at least 48 hours to generate a useful report. Newborn babies are too delicate to give those few hours to the treating clinicians. Moreover, culture is not free from error because of the low yield caused by insufficient sample volumes, intermittent or low-density bacteremia, or suppression of bacterial growth by earlier (i.e., intrapartum) antibiotic administration[1]. It can be falsely sterile, as suggested by postmortem cultures [2]. So, practically blood culture gives an underrepresentation of truly infected newborn. In this situation, current recommendation is initiation of antibiotic therapy for neonates with clinical signs or risk factors of sepsis before blood culture results are available, but because the clinical signs of early onset neonatal sepsis (EONS) are often non-specific, empiric antibiotic therapy may result in the treatment of as many as 30 uninfected neonates for each truly infected baby[3].Therefore,neonatologists have a critical need for laboratory tests that aid in the early diagnosis of neonatal sepsis with a high degree of sensitivity so that no unfortunate incidence morbidity and mortality will occur due to under treatment.

On the same time specificity of the laboratory tests are equally important so that over enthusiastic treatment can be avoided. Various studies have shown that hematological parameters are simple, quick and cost effective tool in the early diagnosis of neonatal sepsis[4,5]. They are the simple tests which can be performed within a short time and can help taking critical decision regarding initiation of antibiotic therapy. So, the present study is undertaken to evaluate the role of haematological parameters in diagnosis of EONS in a tertiary care centre of eastern India.

Material and Methods: Study design: This was a hospital based prospective study. Study area: Study was carried out in the Neonatal Care Unit, Department of Paediatrics and Department of Microbiology of a tertiary care centre for eastern part of Bengal.

Study population: All newborns with clinical suspicion or risk factors for sepsis were recruited into the study. Blood samples of newborn, age less than 72hours with clinical signs of sepsis, including lethargy, refusal to feed, respiratory distress, abdominal distension, vomiting, hypothermia, hyperthermia etc born inside this hospital as well as referred from different centres were enrolled in this study. Maternal fever, foul smelling liquor or prolonged rupture of membranes (>24 hrs) were considered as risk factors for sepsis[6].

Exclusion criteria: Babies who had received antibiotics before collection of blood samples, having surgical problems, chromosomal or congenital anomalies were excluded from the study.

Study period: March 2011 to February 2013

Study was carried out after getting permission from Institutional Ethical Committee.

Procedure: Neonates, who were clinically suspected to have sepsis within the first 72hours of life, were subjected to hematological screening and blood culture.

The hematological screening parameters, included in this study were C- reactive protein (CRP), micro-erythrocyte sedimentation rate (ESR), total leukocyte count (TLC), Immature to Total neutrophil ratio (I:T ratio) and platelet count. These data were collected from the Pathology department except CRP.

C-reactive protein was estimated semi quantitatively using the CRP latex kit manufactured by the Tulip Laboratories (P) Limited. The CRP latex reagent was standardized to detect serum CRP level at or above 6 mg/L, which was considered the lowest concentration of clinical significance. The results were read using the positive and negative controls as reference for agglutination. Visible agglutination of latex particles constituted a positive result which indicated a level of CRP≥6 mg/L. Semi quantitative result was generated after making serial dilution as per manufacturer guideline.

C-Reactive protein (CRP): >10mg/L, Micro ESR (m-ESR) :> 15 mm in the 1st hour, Total leucocyte count (Leucopenia) :< 5,000cells/cu.mm, Immature to total neutrophil count ratio (I:T ratio)> 0.2 , Platelet count:< 1.5 lakhs/cu.mm were considered to be abnormal[6,7]. Positive blood culture was considered as the "Gold Standard" against which the performance of CRP and other haematological parameters were compared. CRP level ≥12mg/L was considered positive for sepsis in our setting as the kit was unable to detect the exact value of 10mg/L.

For blood culture 1 ml of blood was collected maintaining proper aseptic technique and inoculated in 5 ml of brain heart infusion broth with 0.025 % sodium polyanethol sulfonate. The broth was incubated aerobically at 37^0 C. A blind subculture was done after overnight incubation. Inoculated blood culture media were considered negative if there was no sign of growth after continuous incubation for up to 7 days, subcultures being made at 48 hrs, 72 hrs and on 7^{th} day. Media used for subculture were 5% Sheep blood agar, chocolate agar and MacConkeys agar (Himedia Laboratories).Isolates were identified by colonial morphology, Gram staining as well as standard biochemical tests. Antimicrobial susceptibility was performed by Kirby Bauer disc diffusion method following CLSI guidelines[8].

The results of laboratory investigations and other relevant data such as age, sex, birth weight and gestational age as well as symptoms present and risk factors for sepsis of recruited babies were recorded in a proforma.

The results were analysed and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CRP and hematological parameters were calculated. Chi square test was applied to determine P value of the tests and P value <0.05 was considered as significant.

Result: During this two year study period 238 blood samples were obtained from clinically suspected cases of early onset neonatal sepsis. Blood culture was positive in 93(39.07%) cases, of which 3 (1.2%) were non albicans Candida and remaining 90(37.8%) were bacteria. All the culture positive cases were monomicrobial. Infection was found to be more common in male newborn with

male and female ratio of 1.37:1. 71.11% (64/90) of septicemic newborns were low birth weight. Incidence of sepsis in term baby was 28.46% whereas incidence in preterm babies was 49.07%.Relationship of sepsis with birth weight and gestational age was given in Table no. 1 and 2 respectively.

Table:1 Relation between birth weight and sepsis

Birth weight	Sepsis	Non sepsis	Total
Low birth weight <2500gm	64	27	91
Normal birth weight ≥2500gm	26	121	147
Total	90	148	238

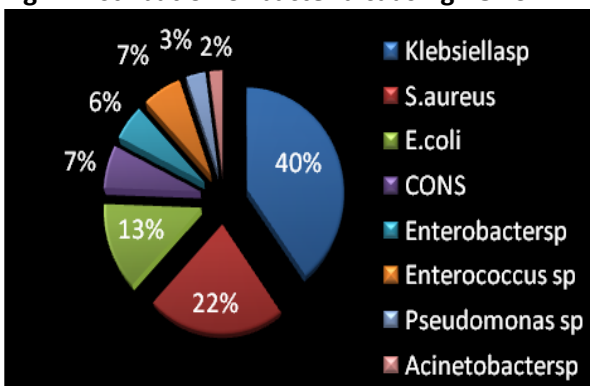
Table:2 : Relation between gestational age and sepsis

Gestational age	Sepsis	Non sepsis	Total
Preterm <36wk	53	55	108
Term ≥36 wk	37	93	130
Total	90	148	238

Gram negative bacteria were the most common organisms isolated in present study accounting 64.44 % (58/90) of total isolated bacteria. *Klebsiella* spp (40%) was the most common organism causing early onset neonatal sepsis followed by *Staphylococcus aureus (*22.22%), *Escherichia coli* (13.33%), *Enterococcus* spp (6.67%), *Enterobacter* spp(5.56%), coagulase negative Staphylococcus(CONS) (6.67%),*Pseudomonas* spp (3.33%) and *Acinetobacter* spp (2.22%). 66.67% (60/90) growth occur after 24hrs and 84.44% (76/90) growth occur within 48 hrs of aerobic incubation. Distribution of bacteria causing EONS was given in Fig No. 1.

In this study CRP was positive in 83.3% of culture proven septicemic newborns. CRP positivity was maximum with CONS, *Enterobacter* and *Acinetobater* sepsis(100%), followed by *Klebsiella* (86.11%), *E.coli* (83.33%), *Enterococcus* (83.33%). Micro ESR, I:T ratio >0.2, abnormal WBC count, Platelet count were positive in 73.33%, 67.78%, 63.33%, 42.22% cases of EONS respectively. Numbers of positivity of hematological parameters were variable with different organisms that were shown in Table No. 3

Fig: 1 Distribution of bacteria causing EONS



Among the parameters CRP and MicroESR showed high specificity of 91.89% and 90.54% respectively. But positive predictive value of the tests ranges from 86.21% to 48.72%.Performance of different hematological profile was depicted in Table No 4. Value of all the hematological screening parameters were found statistically significant producing P value <0.05.

Discussion: Incidence of culture positive EONS varies from 16.4% to 68.93%[4,5,9] in India. Present study it was found to be 37.8% (90/238). This variation may be due to consideration of EONS up to 72 hrs or 7 days or taking single or double specimen for culture might be a cause of this variation.

70.33% (64/91) of LBW babies were suffered from sepsis confirming that low birth weight served as a predisposing factor of sepsis. This result was similar to the studies in elsewhere in India[5, 10]Male newborns were worst hit by the infection, with male and female ratio of 1.37: 1. This male dominance was supported in almost all studies[4,5,9, 11].

The most frequent offender of EONS was *Klebsiella* spp(40%) followed by *S.aureus* (22.22%),*Escherichia coli*(13.33%), *Enterococcus* spp (6.67%), *Enterobacter* spp (5.56%), CONS (6.67%), *Pseudomonas* spp (3.33%) and *Acinetobacter* spp (2.22%) etc. This was similar to the observation published in National Neonatal Perinatal Database[12]and in studies conducted in Pune [4] Wardha[5,11].

Table 3. Organism Wise Distribution Of Hematological Parameters In Culture Positive Early Onset Sepsis

Blood culture organisms(n)	Cases with raised CRP ≥12mg/L		Cases with raised micro ESR		Cases with I:T ratio>0.2		Cases with WBC count<5000/ cu.mm		Cases with abnormal platelet count	
	n	%	n	%	n	%	n	%	n	%
Klebsiella sp(36)	31	86.11	28	77.78	27	75.00	24	66.67	16	44.44
S.aureus(20)	15	75.00	15	75.00	12	60.00	15	75.00	9	45.00
E.coli(12)	10	83.33	8	66.67	9	75.00	9	75.00	5	41.67
CONS(6)	6	100.00	4	66.67	3	50.00	2	33.33	3	50.00
Enterobacter sp(5)	5	100.00	3	60.00	4	80.00	3	60.00	2	40.00
Enterococcus sp(6)	5	83.33	4	66.67	4	66.67	2	33.33	3	50.00
Pseudomonas sp(3)	1	33.33	2	66.67	1	33.33	1	33.33	0	0.00
Acinetobacter sp(2)	2	100.00	2	100.00	1	50.00	1	50.00	0	0.00
Total(90)	75	83.33	66	73.33	61	67.78	57	63.33	38	42.22

Table: 4 Performance of Hematological markers of sepsis

	Sensitivity	Specificity	PPV	NPV	Statistics
CRP	83.33%	91.89%	86.21%	90.07%	x^2= 133.332 with df 1,P <0.000
Micro ESR	73.33%	90.54%	82.50%	84.81%	x^2= 99.482 with df 1,P <0.000
I:T ratio >0.2	67.78%	89.19%	79.22%	81.99%	x^2= 80.405 with df 1,P <0.000
Abnormal WBC count	63.33%	81.08%	67.06%	78.43%	x^2= 46.171 with df 1,P <0.000
Abnormal platelet count	42.22%	72.97%	48.72%	67.50%	x^2=5.196 with df 1,P <0.02

PPV= Positive Predictive Value, NPV= Negative Predictive Value, x^2= Chi square , df= degree of freedom

But some differences in bacteriological profile had been found in studies done in different region of India [9, 10, 13] as well as abroad[14].

C - reactive protein is the most well studied acute phase reactant in the neonatal sepsis. In present study CRP was able to identify all cases of EONS caused by CONS*, Enterobacter, Acinetobacter* and almost all cases of caused by *Klebsiella* spp (86.11%), *E.coli* (83.33%), *Enterococcus* spp (83.33%). Similar observation was revealed in a study in Nigeria [3].

In this study CRP has sensitivity of 83.33% but in some Indian studies sensitivity of CRP was just 16.9%[9], 48.39% [5],50% [13] and 52.3% [11]whereas in the studies in Thailand[15], Pakisthan[16] and Nigeria[3] sensitivity of the test was 100%,85.6%, and 74% respectively. One of the causes of the wide variation is the difference in laboratory techniques (Latex agglutination, ELISA, RIA, Nephelometry, Immunoturbidimetry) employed for the test. Sample size also act as a deciding factor for this discripancy.CRP appeared most useful test in our situation having high specificity and negative predictive value. Elevated CRP in relation to sepsis was found to be highly significant (P value<0.001) in this study.

In present study Micro ESR also showed a high specificity 90.54% in compared to the studies conducted by different researchers[5, 11,13]. In a study in Pune[4] 72.73% specificity was observed. But it was difficult to rely on the micro ESR alone as it might spuriously be high in neonates with haemolysis and low in newborns with disseminated consumptive coagulopathy[7].

Abnormal WBC count was found to be the less specific and sensitive than that of I:T ratio>0.2. This was in accordance with a study in Pune, India[4]. Result of present study was in contrast to a study done by S.K Anwer et al [17] they observed that abnormal WBC count was the most specific test with better PPV and NPV than I:T ratio. But

sensitivity of WBC counts was only 14.28% in studies in Karachi[17], Chennai[13] and 8.39% in Wardha[5], whereas it was 63.33% in present study and 89.23% in a study in Pune [4].

Thrombocytopenia was found to be poor predictor of EONS in this study and the result was also supported by the findings of some other Indian studies [5,9,10,11]. Low yield might be due to the fact that platelet counts are usually low in newborns in the first week of life.

The variation in the results of these parameters in different studies might be due to difference in the blood sampling time, exact method of test employed, the diagnostic criteria followed, the severity of infection and the age of the neonates.

Result of all the haematological parameters including CRP must be evaluated in the light of the clinical condition of the newborn. Truly speaking, if there is already strong clinical suspicion of the infection, the test result would not change the decision of initiating treatment. Similarly, the neonatologist might not start treatment in absence of clinical conditions suggestive of infection even if the tests were positive. The result of these tests is most required in situation when the case history and the condition of the neonates put the clinician in serious dilemma about the diagnosis. However, the most critical point for the neonatologist while evaluating a newborn is to rule out sepsis. Value of these simple and easy to perform hematological tests was more for excluding the diagnosis of neonatal sepsis. Moreover, a great advantage of this test was result usually remains unaltered by prior antibiotic therapy[13]. A lacuna of this study was inability to detect CRP value between 10 to 12mg/L so sensitivity of the test might be more than the observed value.

Conclusion: After vivid evaluation the parameters of sepsis, CRP levels appeared to be the single best predictor for diagnosing EONS in comparison to other hematological parameters. Semi quantitative assay of CRP is simple as well as easy to perform at the bedside by any medical staff and result will be available within 10min. However, sensitivity of 83.33% is unacceptably low for making critical decisions of sepsis but its high negative predictive value if considered along with a good clinical judgment, this test might reduce unnecessary antimicrobial therapy which could otherwise give rise to the emergence of resistant strains of pathogen and put these sick newborn at risk for allergic and adverse side-effects with increased hospitalization costs.

A few new markers, like interleukin (IL-6), procalcitonin (PCT) appear to show considerable promise as a diagnostic test for neonatal sepsis.

Acknowledgment: We want to acknowledge the immense help received from the Laboratory Technicians of Department of Microbiology, Pathology and faculty members of Department of Paediatrics while performing the work.

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Conflict of interest: None

Funding: None