Clinical correlation of neonatal and maternal hematological parameters as predictors of neonatal sepsis

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ABSTRACT

Background: Neonatal sepsis is one of the major causes of neonatal mortality in the developing countries contributing to 15% of all neonatal deaths. The yield of a positive blood culture ranges from 8-73%. Moreover, the technique of blood culture is time consuming and demands a well equipped laboratory which is not available in most of the community hospitals. Therefore, the need is for a test that is cheap, easily performed with quick availability of reports.

Aim: To determine haematological determinant predicting sepsis in neonates.

Methods: A prospective study carried out in the tertiary hospital during during July 2011- December 2011. Thirty new born babies with signs and symptoms of septicemia (aged 0 to 28 days) were included in the study. Hematological scoring was done by adopting Manroe and Rodwell et al scoring system on neonatal blood and compared with maternal hematological parameters with statistical analysis.

Results: 30 cases with clinical diagnosis of neonatal sepsis were analyzed. The maternal risk factor associated with neonatal sepsis in this study was premature rupture of membranes with variable duration in 66.66% of cases and in one case there was foul smelling liquor. Analysis of individual hematological parameters reflects that total polymorphonuclear leucocytes count had sensitivity of 50%, NPV 88.8% (p - .004). Total immature PMN count had sensitivity of 50%, and negative predictive value of 90.9%. The sensitivity of band cells percentage in the present study is 50% and NPV 90.9%. The hematological parameters in the mother of neonates with sepsis showed elevation of total leucocyte count > 12,000 / cu.mm in 23.3% of cases.

Conclusion: A hematological score of 3-4 with CRP>10mg/L in blood culture negative cases would be an effective guideline to make decisions regarding judicious use of antibiotic therapy. These tests are simple, quick, cost effective and readily available tool in diagnosing neonatal sepsis.

Key words: hematological scoring, maternal parameters, neonatal sepsis

INTRODUCTION

Neonatal septicemia is one of the major causes of neonatal mortality in the developing countries contributing to 15% of all neonatal deaths.[1] Though, it is a life-threatening condition, yet treatable if diagnosed early. Unfortunately, the early warning signs and symptoms are often nonspecific and can easily be confused with those from non infective causes and pose difficulty in establishing an early clinical diagnosis.[2] The antibiotic therapy is usually initiated based on the clinical suspicion which sometimes result in overtreatment and eventually leading to emergence of multiresistant organisms. Blood culture is still considered to be the 'gold standard' for diagnosis of septicemia; however, its accuracy has been questioned because of spurious positive results due to contamination and negative blood cultures in fatal generalized bacterial infections. The yield of a positive blood culture ranges from 8-73% as shown in various studies.[3] However; the limiting factors are time consumed as well as requirement of a well equipped laboratory which mostly is not available at community level hospitals.[4]

In recent past, various investigators have evaluated some highly sensitive and specific inflammatory markers (eg. ELISA methods, haptoglobins, interleukins and counterimmunoelectrophoresis etc,) to diagnose neonatal sepsis.[5] Indeed these

markers are sensitive and specific, but are sophisticated and expensive. Various cheap but reliable laboratory tests have been evaluated for the diagnosis of systemic infection in neonates.[6] The complete blood count (CBC) with the various neutrophil parameters and C-reactive protein (CRP) are the most frequently used.[7] It is therefore a need arises for a test that is cheap, have maximum sensitivity and specificity; can be performed easily and reports made available quickly.

The present study aims to evaluate the neonatal and maternal clinical manifestations and their hematological parameters, individually and in combination, as parameters which can be used to formulate a scoring system in determining neonatal sepsis. This is a simple bed-side test which can be done within a short time before putting the neonate on antibiotic therapy.

METHODOLOGY

This prospective study was carried out at a tertiary care hospital during July –Dec'2011. Neonates with RDS, cyanosis, apnea, pneumonia, low APGAR score, birth asphyxia, lethargy, hypoglycemia, temperature instability and seizures as well as those neonates with maternal history of infections such as premature rupture of membranes, pneumonia, upper respiratory tract infections, urinary tract infections and vaginitis were included in this study. Newborn with gestational age of 30 or more weeks and weight of more than or equal to 1000 gm were part of the study.

Careful history taking was done and the information was recorded in a specified proforma designed by the investigator. Observing aseptic precaution 2ml blood was withdrawn from suspected sepsis subjects within 24 hours of admission. 1ml sample was anticoagulated with EDTA and by using Sysmex, model KX-21 automated Haematology analyzer, values of total leucocyte count and platelets were noted.

Peripheral blood smears were stained by Leishman stain. Differential leucocyte counts (DLC), total neutrophil count(TNC),immature neutrophil count(I) and mature neutrophil count (M) were performed. IT ratio (Immature to total neutrophil) and IM ratio (immature to mature neutrophils) ratio were calculated. IT ratio is calculated dividing the total immature count by total neutrophil count (including both mature and immature neutrophil count).Degenerative changes (toxic granulation,vacuolation and Dohle bodies were graded from 1 to 4. According to Zipusky et al., toxic granulation was graded as 0 or (-) which indicated normal granulation or no toxic granules seen, (+) slight, (++) approximately 50% of neutrophils contained dark granules, (+++) very high granulation in most cells, and (++++) gross toxic granulation with the nucleus obscured by toxic granules.

Another 1ml of blood sample was inoculated aseptically into conventional blood culture bottle (Brain Heart Infusion broth bottle) for culture of the organisms and sent to the microbiology department. Simultaneously blood was sent for estimation of CRP.

2ml of maternal blood was collected in EDTA and the total leucocyte count and platelet count was recorded using Sysmex, model KX-21 automated Haematology analyzer.

Statistical analysis:

Sensitivity, Specificity, Negative predictive value, Positive predictive value and p value using chi-square test was calculated for each individual hematological parameter and the hematological score. Rodwell et al, [8]formulated a scoring system in their study based on normal values, defined by Manroe et al., Hematological scoring system adapted in this study was: If no mature PMNs seen on blood film, score 2 rather than 1 for abnormal.

Points	Abnormality	Scoring
Total WBC count <5000/mm3 or ≥ 25,000, 30,000 and 21,000/mm3 at birth,12-24 hrs and day 2 onward	Decreased or increased	1
Total PMN count 7,800 – 14,500/mm3(< 72 hrs) 1750-4500/mm3(>72 hrs)	Decreased or increased	1
Immature PMN count 500-1450 (<72 hrs) 500(upto 28 days)	Increased	1
I:T ratio (>0.2)	Increased	1
I:M ratio	≥ 0.3	1
Platelet count/mm3	≤ 100,000/mm3	1
Degenerative changes in PMN	≥ 3+	1

Interpretation

Score	Interpretation
≤2	Sepsis is very unlikely
3 or 4	Sepsis is suspected
≥5	Sepsis or infection is very likely

RESULTS

Thirty cases with clinical diagnosis of neonatal sepsis were analyzed. The maternal risk factor associated with neonatal sepsis in this study was premature rupture of membranes with variable duration in 66.66% of cases and in one case there was foul smelling liquor. Neonatal infections are more common within seven days of birth. Neonatal sepsis was predominantly seen in males (63.3%). 26.6% were preterm and 73.3% were term deliveries. In 73.3% of neonates the weight was appropriate for gestational age and in 26.6% of cases the weight was less for gestational age. In 60% of cases the mode of delivery was cesarean section and in 40% of cases normal delivery.[Table 1]

Table. 1. Association of neonatal profile with neonatal sepsis-30

Patient's Profile	Blood Culture +VE N=2	Blood Culture -VE N=28	Total N=30	%
Preterm	-	8	8	26.6
Full Term	2	20	22	73.3
Male	1	18	19	63.3
Female	1	10	11	36.6
Weight				
AGA	2	20	22	73.33
SGA	-	8	8	26.6
Manner Of Delivery				
LSCS	1	17	18	60
NVD	1	11	12	40

The most common presenting symptom of neonatal sepsis observed in the present study were respiratory distress syndrome in 60%, poor cry/ poor feeding in 26.6%, pneumonia, meconium aspiration in 3.33% and seizures in 6.66% of cases. The two cases with proven neonatal sepsis on blood culture presented with seizures.[Table2]

Table. 2. Association of neonatal clinical symptoms and maternal symptoms with neonatal sepsis-30

Clinical Profile	Blood Culture +Ve N=2	Blood Culture -Ve N=28	Total N=30	%
APGAR SCORE				
1-3 min	-	-	-	-
4-6 min	-	13	13	43.3
7-9 min	2	15	17	56.6
NEONATAL SYMPTOMS				
RDS	-	18	18	60
POOR CRY/POOR FEEDING	-	8	8	26.6
PNEUMONIA	-	1	1	3.33
MECONIUM ASPIRATION	-	1	1	3.33
SEIZURES	2	-	2	6.66
MATERNAL RISK FACTORS				
+	-	21	21	70
-	-	9	9	30

In the present study out of 30 cases with neonatal sepsis blood culture was positive only in two cases (6.66%). On analysis of individual hematological parameters in the present study the sensitivity of total leucocyte count was 50%, specificity 67.8% with PPV 10% and NPV of 95%. Total polymorphonuclear leucocytes count had sensitivity of 50%, specificity 28.55,PPV 4.76% and NPV 88.8% with a p value of .004. Total immature PMN count had sensitivity of 50%, specificity 35.7%,PPV 5.26% and negative predictive value of 90.9%. The sensitivity of band cells percentage in the present study is 50%, specificity 35.7%, PPV 5.26% and NPV 90.9% .[Table 3]

Table. 3. Association of neonatal haematological parameters with neonatal sepsis

Hematological Parameters	Blood Culture +Ve N=2	Blood Culture –Ve N=28	Total N=30	p
TLC/MM^3				
Abnormal	1	9	10(33.3%)	.>0.05(NS)
Normal	1	19	20(66.6%)	
TOTAL PMN/MM^3				
Abnormal	1	20	21(70%)	.004(S)
Normal	1	8	9(30%)	
IMMATURE PMNS				
Abnormal	1	18	19(63.3%)	
Normal	1	10	11(36.6%)	>0.05(NS)
PLATELET COUNT				
Less than 1 Lakh	1	9	10(33.33%)	>0.05(NS)
Normal	1	19	20(66.6%)	
I/T RATIO				
<0.2	2	28	30(100%)	
>0.2	-	-	-	
I/M RATIO				
<0.3	2	28	30(100%)	
>0.3	-	-	-	

In the present study I/T ratio and I/M ratio did not contribute to the diagnosis of neonatal sepsis. The platelet count of ≤ 1 lakh /cumm showed sensitivity of 50%,specificity of 67.8%,PPV of 10% and NPV of 95% with p value of <.05. In the present study haematological score 3-4 was seen in 30% and rest of the neonates with clinical suspicion of sepsis scored 2.The sensitivity was 22.2%, specificity 66.6% PPV 22.2% and NPV 66.6%.[Table 4]

Table.4. Performance of individual hematological parameters in cases of neonatal sepsis

Hematological Test	Senstivity %	Specificity %	Positive Predictive Value%	Negative Predictive Value%
TLC/mm3	50	67.8	10	95
TOTAL PMNs/mm3	50	28.5	4.76	88.8
IMMATURE PMNs	50	35.7	5.26	90.9
PLATELET COUNT < 1 LAKH	50	67.8	10	95
Sepsis suspected (Score 3-4)	22.2	66.6	22.2	66.6

In the present study CRP >10mg/L was the cut of value to suspect neonatal sepsis and was positive in 12 cases (40%).The sensitivity was 100%, specificity was 93.3%, PPV 14.2% and NPV of 100%.In cases where the blood culture is negative CRP > 10 mg/l can be considered as a criteria for proven sepsis. Out of the 30 cases analyzed the hematological parameters in the mother of neonates with sepsis showed elevation of total leucocyte count > 12,000 / cu.mm in 23.3% of cases. The platelet count was within normal limits. All of them had good antenatal care and the infections were adequately treated prior to delivery.

DISCUSSION

The early signs of sepsis in the newborn are nonspecific. A high index of suspicion is important. Early diagnosis of neonatal septicemia is primarily based on clinical evaluation. Laboratory diagnosis needs battery of investigations as no single investigation is a very good indicator of sepsis including blood

culture which is considered a gold standard.

Infection occurring at less than 72 hours of age are usually caused by bacteria acquired in utero or during delivery, whereas infection after that time most likely have been acquired after birth. Thus it is essential to know the maternal illnesses which can predispose to neonatal sepsis. The maternal risk factors associated with neonatal sepsis in this study were PROM with variable duration in 66.66% of cases and in one case there was foul smelling liquor. Due to good antenatal care and adequate treatment of maternal infections, the maternal risk factors did not contribute much to the causation of neonatal sepsis.[9]

Neonatal infections are more common within seven days of birth which was also observed in this study. Neonatal sepsis was predominantly seen in males (63.3%), which is comparable with other studies. This is possibly due to impaired defence mechanism and low immunoglobulin G levels in male children. The factor regulating the synthesis of globulin is situated on the X chromosome. Male has only one X chromosome; hence males are less immunologically protected than the females. Preterm neonates are more susceptible to infection due to low levels of IgG and relative deficiency of IgM making them more prone to gram negative infections. The preterm neonates have less effective phagocytosis and chemotactic activity, therefore rapid invasion of the offending organism. In the present study, 26.6% were preterm and 73.3% were term deliveries. In 73.3% of neonates the weight was appropriate for gestational age and in 26.6% of cases the weight was less for gestational age.[10] In 60% of cases the mode of delivery was caesarean section and in 40% of cases normal delivery.

The mortality in sepsis is very high if not treated in time; it compels physicians to initiate treatment based mainly on clinical suspicion even without waiting for confirmation of positive blood culture. Because of the low positivity of blood culture and its unavailability in peripheral health centres and the time allotted for the result to be obtained, there is a need for other simpler tests.[9] The complete blood count, CRP and band cell count is widely used either singly or in combination. The hematological criteria of Manroe and Rodwell et al, for neonatal sepsis were the most reliable of the published criteria evaluated in literature, which could distinguish sepsis and probable sepsis. In the present study out of 30 cases with neonatal sepsis blood culture was positive only in two cases (6.66%).The organisms isolated were pyococcal meningitidis and candida albicans.

On analysis of individual hematological parameters in the present study the sensitivity of total leucocyte count was 50%, specificity 67.8% with PPV 10% and NPV of 95%. So observation from this study showed that total leucocyte counts acts as a good parameter for confirmation of

sepsis. Neutropenia has been more common in association with sepsis, compared to neutrophilia, probably because of increased adherence to altered endothelial cells and utilization at the site of infection. In this study, total polymorphonuclear leucocytes count 1750-5400 cells/mm^3(>72 hrs) and 7800-14500 cells/mm^3(<72 hrs) had sensitivity of 50%,specificity28.55,PPV 4.76% and NPV 88.8% with a p value of .004.In this study the total PMNs was associated with low positive predictive value and low specificity with significant p value. Therefore it should not be used in isolation as a predictor of sepsis.[10]

A shift to the left in differential white cell count with a raised immature neutrophil count (band form) has been documented in patients with bacterial infection. In the present study total immature PMN count with cut off value 500-1450 cells/mm^3(<72 hrs of age) and > 500 cells/mm^3(>72 hrs) had sensitivity of 50%,specificity 35.7%,PPV 5.26% and negative predictive value of 90.9%.Despite a significant rise in immature neutrophil count in neonates with suspected infection, various cut of values were examined which gave low specificity and large number of false positive results. Therefore this parameter alone should not be evaluated for diagnostic purpose. The sensitivity of band cells percentage in the present study is 50%, specificity 35.7%, PPV 5.26% and NPV 90.9% .These results were similar to the observations of studies of Ghosh et al [11], Rodwell, and Lesilie et al.[8]

In the present study I/T ratio and I/M ratio did not contribute to the diagnosis of neonatal sepsis. Neonates with sepsis develop thrombocytopenia possibly because of damaging effects of endotoxin on platelets, or due to disseminated intravascular coagulation. Platelet count of ≤ 1 lakh /cumm was considered as cut off value with sensitivity of 50%, specificity of 67.8%, PPV of 10% and NPV of 95% with p value of <.05.The sensitivity and NPV was comparable with other studies. This parameter could be used as an early but nonspecific marker for sepsis.[12]

As no single individual hematological parameter is superior compared to other in predicting neonatal sepsis; however, a combination of these parameters in the form of Hematological scoring system (HSS) has been recommended. The score adapted in this study was of Rodwell et al and Manroe et al.[7,8] A score of ≤ 2 sepsis is very unlikely, 3-4 sepsis is suspected, and ≥ 5 sepsis or infection is very likely. In the present study score 3-4 was seen in 30% and rest of the neonates with clinical suspicion of sepsis scored 2.The sensitivity was 22.2%, specificity 66.6% PPV 22.2% and NPV 66.6%.These low percentages was probably due to small sample size. Considering the relatively high specificity, positive predictive value this study implies that hematological score was more reliable as a screening tool for sepsis than any of the individual hematological parameter.

In the present study CRP >10mg/L was the cut of value to suspect neonatal sepsis and

was positive in 12 cases (40%).The sensitivity was 100%, specificity was 93.3%, PPV 14.2% and NPV of 100%. In cases where the blood culture is negative CRP > 10 mg/l can be considered as a criterion for proven sepsis.

Out of the 30 cases analyzed the hematological parameters in the mother of neonates with sepsis showed elevation of total leucocyte count > 12,000 in 23.3% of cases. The platelet count was within normal limits. All of them had good antenatal care and the infections were adequately treated prior to delivery.

Considering high mortality and morbidity associated with sepsis, tests with high sensitivity and NPV are most desirable. In the present study the following tests in neonates had high negative predictive value with equal sensitivity.[12] They in the order of priority are total leucocyte count, platelet count, immature PMN and total PMN. The specificity of total leucocyte count and platelet count is high in comparison to other tests. A hematological score of 3-4 with CRP>10mg/L in blood culture negative cases would be a effective guideline to make decisions regarding judicious use of antibiotic therapy which will be life saving, provide early cure, reduce mortality, shorten hospital stay and as well as minimize the emergence of resistant organism due to misuse of antibiotics. These tests are simple, quick, cost effective and readily available tool in diagnosing neonatal sepsis.

CONCLUSION

High mortality and morbidity associated with sepsis it is pertinent to look for tests with high sensitivity and NPV for screening and diagnosis. It would be of great importance regard to judicious use of antibiotic therapy which will be life saving and as well as minimize the emergence of resistant organism due to misuse of antibiotics. These tests are simple, quick, cost effective and readily available tool in diagnosing neonatal sepsis.

AUTHOR NOTE

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