HAEMATOLOGICAL SCORING SYSTEM (HSS) IN EARLY DIAGNOSIS OF NEONATAL SEPSIS: A STUDY FROM TERTIARY CARE HOSPITAL FROM NORTH EAST INDIA

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ABSTRACT

BACKGROUND

Neonatal sepsis is defined as a clinical syndrome of bacteraemia with systemic signs and symptoms of infection in the first 4 weeks of life. Timely diagnosis of sepsis in neonates is critical as the illness can be rapidly progressive and in some instances fatal. The definitive diagnosis of septicaemia is made by a positive blood culture, which requires a minimum of 48-72 hours with a positive result in only 10-60% of cases. Haematological parameter can be evaluated for the early diagnosis of neonatal bacterial infection.

AIMS

The current study was undertaken to study the haematological parameters for early diagnosis of neonatal septicaemia using Rodwell's scoring system with the aims and objectives of evaluating the Haematological Scoring System (HSS) in early diagnosis of neonatal sepsis and to find out its significance.

MATERIALS AND METHODS

Prospective Hospital based cross-sectional study, carried out in the clinical haematology section of the Department of Pathology, Assam Medical College and Hospital (AMCH) during the period of August 2013 to July 2014 with neonates suspected to have sepsis admitted in Neonatal Intensive Care Unit, (NICU). A total of 210 neonates suspected of having sepsis were enrolled in this study. Ethical Clearance for the study was obtained from Institutional Ethics Committee, AMCH, Dibrugarh, Assam and Informed written consent from legal guardians were obtained. Relevant maternal and neonatal histories were recorded in a predesigned and pretested proforma. Blood samples (2 mL) were collected from the peripheral venous puncture within 24 hours of admission before initiation of antibiotic therapy and all necessary parameters were evaluated.

STATISTICAL ANALYSIS

The data obtained was tabulated using Microsoft Excel and tested through Chi-square test and Fisher Exact test when the expected frequencies are less than 5.

RESULTS AND OBSERVATIONS

The mean age of the study population was 2±1 days. In the present study, 12.86% of the neonates had birth weight <1500 g. Tachypnoea were the commonest complaints. The blood culture was negative in 77.15% (162/210) of neonates and positive in 22.85% (48/210) of neonates where Gram negative organisms were seen in 62.5% (30/48) of the neonates and Gram positive infection in 37.5% (18/48). Our study observed that score of ≥4 was reliable as a screening tool for sepsis than any of the individual haematological parameter. Among the variables of HSS it was observed that raised I:T ratio was highly sensitive in picking neonatal sepsis, while degenerative changes showed least sensitivity but higher specificity which was statistically significant ($p < 0.05$).

CONCLUSION

The early diagnosis of neonatal sepsis with the help of HSS may provide a guideline to decisions regarding antibiotic therapy and hereby minimize the risk of emergence of resistant organisms due to misuse of antibiotics.

KEYWORDS

Haematological Scoring System, Neonatal Sepsis, Infections.

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INTRODUCTION

Neonatal sepsis is defined as a clinical syndrome of bacteraemia with systemic signs and symptoms of infection in the first 4 weeks of life.[1] When pathogenic bacteria gain access into the blood stream, they may cause overwhelming infection without much localization or may get predominantly localized to the lung or the meninges.[2]

Neonatal sepsis is responsible for about 30-50% of the total neonatal deaths in developing countries.[3] Though, it is a life-threatening condition, yet treatable if diagnosed early.

It is a vexing problem because of its nonspecific clinical picture.[4], which makes it difficult to establish an early clinical diagnosis. Newborns, especially the premature are prone to serious infections, because the signs of these infections may be absent or minimal and hard to detect. Thus, fatal septicaemia may occur with little warning.[5] Timely diagnosis of sepsis in neonates is critical as the illness can be rapidly progressive and in some instances fatal.[6]

The overall incidence of neonatal sepsis occurs between 1 and 8 per 1000 live births. In developing countries, mortality rate is between 11-68 per 1000 live births.[7] Neonatal sepsis can be divided into two subtypes: early and late, depending upon whether the onset of symptoms is during the first 72 hours of life or later.

In the presence of predisposing factors, early clinical suspicion coupled with sepsis screen will detect neonatal septicaemia earlier, which will enable the clinician to treat the infection timely and adequately, which in turn will help to reduce the neonatal morbidity and mortality thus needing a test that is cheap, easily performed with quick availability of reports. The definitive diagnosis of septicaemia is made by a positive blood culture, which requires a minimum of 48-72 hours with a positive result in only 10-60% of cases.[8] But this highly specific microbiologic parameter is usually not available in most of the peripheral health centres and time consuming, therefore haematological parameter can be evaluated for the early diagnosis of neonatal bacterial infection. Thus, the current study was undertaken to study the haematological parameters for early diagnosis of neonatal septicaemia using Rodwell's scoring system with the aims and objectives of evaluating the Haematological Scoring System (HSS) in early diagnosis of neonatal sepsis and to find out its significance.

MATERIALS AND METHODS

This prospective hospital-based cross-sectional study was carried out in the clinical haematology section of the Department of Pathology during the period of August 2013 to July 2014.

Source of Data

Neonates suspected to have sepsis admitted in Neonatal Intensive Care Unit, (NICU), Assam Medical College and Hospital (AMCH), Dibrugarh were studied.

Selection Criteria

The study included neonates with features suggestive of sepsis, neonates with history of recent maternal infection i.e. within 2 weeks prior to delivery [Fever >38°C, foul smelling liquor, premature rupture of membrane (PROM) >24 hours etc.]

Neonates with major congenital anomaly, inborn errors of metabolism, haemolytic jaundice and neonates who received antibiotics before collection of blood samples were excluded.

Sample Size

A total of 210 neonates suspected of having sepsis were enrolled in our study.

Ethical Clearance for the Study was obtained from Institutional Ethics Committee and Informed written consent from legal guardians were obtained.

Method of Collection of Data

The study included neonates aged 0 to 28 days admitted for sepsis evaluation in NICU, Departmental of Neonatology, AMCH, Dibrugarh.

Newborn with less than 37 weeks gestational age were regarded as preterm and more than 37 weeks as term.[9]

Study Procedure

Relevant maternal and neonatal histories were recorded in a predesigned and pretested proforma. Blood samples (2 mL) were collected from the peripheral venous puncture within 24 hours of admission before initiation of antibiotic therapy. Under complete aseptic conditions, 0.5-1 mL of blood sample was obtained. The samples were collected in tripotassium ethylenediaminetetraacetic acid containing non-siliconized vacutainer tubes. Sepsis workup involved complete blood counts along with haematological score (Rodwell's) and microbial culture.

Red blood cell count, haemoglobin, uncorrected WBC count, platelet count were measured using 5 part fully automated Sysmex [XS-800i] analyser. Total leucocyte count readings were corrected for nucleated red blood cells. Peripheral Blood Smears (PBS) were drawn, stained with Leishman stain and examined under oil-immersion lens of light microscope at a magnification of ×1000. Differential counts were performed by counting at least 200 cells. PBS were examined for nucleated red blood cells (N-RBC), total white cell count, total neutrophil count, immature neutrophil count (Including bands and stabs) and mature neutrophil count. Neutrophils were classified as band forms when there were no nuclear segmentation or when the width of the nucleus at any constriction was not less than one-third the widths at its widest portion. Band forms together with less mature cell form were classified as immature Polymorphonuclear (PMN) leukocytes. Using these values, I:M and I:T ratios were computed. Immature neutrophils include promyelocyte myelocyte, metamyelocyte and band form.

One hundred neutrophils were further examined for degenerative changes such as toxic granulations, Döhle bodies and vacuolisations. Degenerative morphologic changes were graded 0 to 4+ according to Zipursky et al.[10] 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.

All the analysis of the PBS findings were reviewed by pathologists blinded to the infection status of the neonates.

The haematological findings were analysed according to the Haematologic Scoring System (HSS) of Rodwell's et al,[11] which includes.

- White Blood Cell (WBC) count and its differential.
- Platelet count, N-RBC (To correct total WBC's count).
- Assessment of degenerative and toxic changes in PMNs.

The HSS assigns a score of one for each of the seven criteria found to be significantly associated with sepsis with

one exception. An abnormal total PMN count is assigned a score of 2 instead of 1 as shown in the [Table 1]. Sensitivity, specificity, positive and negative predictive values will be evaluated for each of the seven criteria of HSS. Blood Culture and CRP estimation were done as per the standard protocol.

Statistical Analysis

The data obtained was tabulated on Microsoft excel spreadsheet and analysed. The data was expressed in terms of rates, ratios and percentages. Effectiveness of various haematological parameters in diagnosing neonatal sepsis were tested using Chi-square test to compare or associate nominal data and Fisher Exact test when the expected frequencies are less than 5. A probability value (p value) of less than 0.05 was considered statistically significant. Sensitivity, specificity, Positive Predictive Values (PPVs) and Negative Predictive Values (NPVs) were calculated for each parameter.

RESULTS AND OBSERVATIONS

In our study, 56.67% of neonates were males and 43.33% were females. The male-to-female ratio was 1.3:1. The commonest age group was 24 to 48 hours with 50.95% of neonates followed by 48 to 72 hours with 23.34% and 0 to 24 hours comprised of 13.33% neonates. The mean age of the study population was 2±1 days. In this study 12.86% of the neonates had birth weight <1500 g, 50.48% of the neonates had birth weight between 1500 and 2499 g and 36.67% of the neonates had birth weight more than 2500 g [Table 2].

Respiratory distress which includes tachypnoea, grunt and chest retraction (any two present) and with only Tachypnoea were the commonest complaints present in 91 and 74 neonates, respectively. The other complaints included Jaundice, Feeding Difficulty, Lethargy, Seizure, Shock, Feeding Intolerance/Vomiting and Bleeding [Table 3]. Majority of the neonates showed more than one symptoms.

In our study, maternal history includes preterm labour in 118 cases, PROM >24 hours in 51 cases and prolonged labour >24 hours in 39 cases. These findings showed overlapping in many cases. Other history suggested Urinary tract infection and Upper respiratory tract infection in 6 and 9 cases respectively [Table 4]. Blood culture was negative in 77.15% (162/210) of neonates and positive in 22.85% (48/210) of neonates where Gram negative organisms were seen in 62.5% (30/48) of the neonates and Gram positive infection in 37.5% (18/48). Gram negative organisms formed the commonest group of infections in which Klebsiella pneumoniae infection [Figure 1] were found to be most common followed by Pseudomonas. In Gram Positive Staphylococcus aureus comprised of 7/18 (38.89%) [Table 5]. On evaluation by Rodwell's scoring system, neonates could be categorized as Sepsis to be unlikely in 76 cases, possible in 73 cases and Sepsis/infection very likely in 61 cases [Table 6].

Our study showed 48 neonates were culture positive, of which 15 neonates had HSS score of ≥6 and 33 had score of less than 6 with p value of <0.001, which is highly significant. Likewise, 42 neonates had HSS score of ≥5 and 6 had score of less than 5 showing p-value of <0.001 [Table 7]. The sensitivity, specificity, PPV and NPV of HSS with cut-off score of 6 in predicting sepsis was 31.25%, 99.38%, 93.75% and 82.99% respectively. Similarly, the sensitivity, specificity, PPV and NPV of HSS with cut-off score of 5 in predicting sepsis was 87.5%, 86.42%, 65.63% and 95.89% respectively. The sensitivity of HSS with cut-off score of 4 and 3 in predicting sepsis was 100% and specificity was 69.75% and 46.91% respectively [Table 8].

47 neonates had raised I:T ratio and 1 had normal I:T ratio (p value is <0.001). The sensitivity of raised I:T ratio in predicting sepsis was found to be 97.92% and specificity 54.32%, PPV 38.84% and NPV 98.88%; 37 neonates had abnormal total PMN count and 11 had normal total PMN count (p value 0.058) showing sensitivity of total PMN count in predicting sepsis as 77.08% and specificity 38.27%, PPV 27.01% and NPV 84.93%. In our study 45 neonates had raised I:M ratio and 3 had normal I:M ratio (p value is <0.001). The sensitivity of I:M ratio in predicting sepsis was 93.75% and specificity 91.36%, PPV 76.27%, NPV 98.01%; 41 neonates had abnormal immature PMN count and 7 had normal immature PMN count (p value 0.006). The sensitivity of abnormal immature PMN count in predicting sepsis was 85.42%, specificity was 35.19%, PPV 28.08% and NPV 89.06%. Of the 48 neonates with sepsis, 27 neonates had abnormal total WBC count and 21 had normal total WBC count (p value <0.001). The sensitivity of abnormal total WBC count in predicting sepsis was 56.25%, specificity was 72.22%, PPV 37.5% and NPV 84.78%; 20 neonates showed degenerative changes [Figure 2], while 28 had no degenerative changes (p value <0.001). The sensitivity of degenerative changes in predicting sepsis was 41.67% and specificity 85.19%, PPV 45.45% and NPV 83.13%. In our study, 34 neonates had platelet count of ≤100,000 mm^3, while 14 had platelet count of >100,000/mm^3 (p value <0.001). The sensitivity of platelet count with cut-off of 100,000 was 70.83% and specificity 59.88%, PPV 34.34% and NPV 87.39%. Of the 48 neonates with sepsis, 36 neonates had platelet count of ≤150,000/mm^3, while 12 had platelet count of >150,000 mm^3 (p value 0.389). The sensitivity of platelet count with cut-off of 150,000 mm^3 was 75% and specificity 31.48%, PPV 24.49% and NPV 80.95%; 18 neonates (18/48) had CRP +ve, while 30 had CRP –ve (p value 0.004). The sensitivity of CRP was 37.5% and specificity was 83.33%, PPV 40.00% and NPV 81.82% [Table 9 and 10].

In the group of probable sepsis, the preterm neonates were 51.2% (83/162), whereas the group with proven sepsis consisted of 72.9% (35/48), which shows a significant correlation between preterm and positive blood culture with p value of 0.007, but no statistically significant difference was observed (p value 0.639) between early [66.67%] and late onset sepsis [33.33%] in neonates. The neonatal profile also showed proven sepsis in 71% of the neonates with low birth weight <2500 grams in comparison to 29% of neonates with birth weight >2500 grams, which is also statistically significant (p- value 0.004) [Table 11].

Criteria	Abnormal Range*	Score
I:T PMN ratio	0.12	0
	Increased > 0.2	1
Total PMN count	Decreased/increased	1
	No mature PMN seen	2
I:M PMN ratio	≤ 0.3	0
	≥ 0.3	1
Immature PMN count	600	0
	Increased	1
Total WBC count	≤5,000/µL	1

	≥ 25,000 at birth	
	≥ 30,000, 12-24 hrs.	
	≥ 21,000 day 2 onward	
Degenerative changes in PMN	≥ 3+	1
Platelet count	≤ 100,000/ µl	1
*Normal values as defined by reference ranges of Manroe et al[12]		
Table 1: Haematologic Scoring System (HSS)		

Score	Interpretation
≤ 2	Sepsis is unlikely
3 or 4	Sepsis is possible
≥ 5	Sepsis or infection is very likely
Interpretation of Haematological Scoring System	

Minimum Score	0
Maximum Score	8

Demographic Profile		Number (n)	Percentage (%)
Sex	Male	119	56.67
	Female	91	43.33
	(Male: Female)	1.3: 1	
Total		210	100
Age (In Days)	0–24 h	28	13.33
	24–48 h	107	50.95
	48–72 h	49	23.34
	72–96 h	16	7.62
	>96 h	10	4.76
Total		210	100
Birth Weight (Grams)	<1500	27	12.86
	1500 G to 2499	106	50.48
	>2500	77	36.67
Total		210	100
Table 2: Demographic Profile of Neonates			

Complaints	Number (n)	Percentage (%)
Respiratory distress	91	43.33
Tachypnoea	74	35.24
Jaundice	30	14.29
Feeding difficulty	12	5.71
Lethargy	12	5.71
Seizure	11	5.24
Shock	9	4.29
Feeding Intolerance/Vomiting	9	4.29
Bleeding	3	1.43
Others	11	5.24
Table 3: Chief Complaints of Neonates		

*Multiple presentations, hence total not shown

History	Number (n)	Percentage (%)
Preterm labour (< 37-week gestational age)	118	56.19
PROM >24 Hours	51	24.29
Prolonged Labour >24 Hours	39	18.57
Foul Smelling Liquor	17	8.1
Others History	12	5.71
Table 4: Maternal History		

Blood Culture			Number (In %)	Total (In %)
Culture Positive n=48	Gram Negative 30/48 (62.51%)	Klebsiella pneumonia	18/30 (60%)	48/210 (22.85%)
		Pseudomonas	6/30 (20%)	
		E. coli	4/30 (13.33%)	
		Klebsiella oxytoca	2/30 (6.67%)	
	Gram Positive 18/48 (37.50%)	Staphylococcus aureus	7/18 (38.89%)	
		Coagulase –ve staphylococcus	6/18 (33.33%)	
		Enterococcus	5/18 (27.78%)	
Culture Negative		n=162		162/210 (77.15%)
Total			**210**	**100%**
Table 5: Culture Profile of Neonates				

Score	Interpretation	Number (n)
≤ 2	Sepsis is unlikely	76
3 or 4	Sepsis is possible	73
≥ 5	Sepsis or infection is very likely	61
Table 6: Interpretation of HSS		

Haematological Score	Culture (+)	Culture (-)	Total (n)	P-value
≥ 6	15	1	16	<0.001
< 6	33	161	194	
≥ 5	42	22	61	<0.001
< 5	6	140	149	
≥ 4	48	49	97	<0.001
< 4	0	113	113	
≥ 3	48	86	134	<0.001
< 3	0	76	76	
≥ 2	48	110	158	<0.001
< 2	0	52	52	
Total	**48**	**162**	**210**	
Table 7: Diagnostic Accuracy of Haematological Scoring System				

Haematological Scoring System	Sensitivity %	Specificity %	PPV %	NPV %
HSS > 6	31.25	99.38	93.75	82.99
HSS > 5	87.5	86.42	65.63	95.89
HSS > 4	100	69.75	49.48	100
HSS > 3	100	46.91	35.82	100
HSS > 2	100	32.1	30.38	100
Table 8: Diagnostic Accuracy of Different Haematological Scores				

Diagnostic Accuracy	I:T Ratio	Culture (+)	Culture (-)	Total (n)	P-value
I:T Ratio	Increased >0.2	47	74	121	<0.001
	Normal	1	88	89	
Total PMN	Decreased/ increased	37	100	137	0.058
	Normal	11	62	73	
I:M Ratio	>0.3	45	14	54	<0.001
	≤0.3	3	148	156	
Immature PMN Count	Raised	41	105	145	0.006
	Normal	7	57	65	
Total WBC	Abnormal	27	45	72	<0.001

	Normal	21	117	138	
Degenerative Changes	Present ≥3+	20	24	44	<0.001
	Absent	28	138	166	
Platelet Count	Reduced ≤100,000/ µL	34	65	99	<0.001
	Normal >100,000/ µL	14	97	111	
Platelet Count	Reduced ≤150,000/ µL	36	111	147	<0.001
	Normal >150,000/ µL	12	51	63	
CRP	Reduced ≤150,000/ µl	36	111	147	0.004
	Normal >150,000/ µl	12	51	63	
Total		**48**	**162**	**210**	

Table 9: Diagnostic Accuracy of Different Haematological Parameters in Predicting Sepsis

Haematological Parameters	Sensitivity %	Specificity %	PPV %	NPV %
I:T Ratio	97.92	54.32	38.84	98.88
Total PMN	77.08	38.27	27.01	84.93
I:M Ratio	93.75	91.36	76.27	98.01
Immature PMN Count	85.42	35.19	28.08	89.06
Total WBC	56.25	72.22	37.5	84.78
Degenerative changes	41.67	85.19	45.45	83.13
Platelet Count (≤100,000/mm3)	70.83	59.88	34.34	87.39
Platelet Count (≤150,000/mm3)	75	31.48	24.49	80.95

Table 10: Association of The Individual Haematological Parameters with Neonatal Sepsis

Patient's Profile	Culture (+) (n=48)	Culture (-) (n=162)	Total	p value
Preterm	35 (72.92%)	83 (51.23%)	118	0.007 <0.05 (Significant) (chi-square test)
Term	13 (27.08%)	79 (48.77%)	92	
Sex				
Male	24 (50%)	94 (58.02%)	118	0.32 >0.05 (Not Significant) (chi-square test)
Female	24 (50%)	68 (41.98%)	92	
Birth Weight				
<1500 gm	13 (27.08%)	14 (8.64%)	27	0.004 < 0.05 (Significant) (chi-square test)
>1500–<2500 gm	21 (43.75%)	86 (53.09%)	107	
>2500 gm	14 (29.17%)	62 (38.27%)	76	
Onset				
Early (<3 Days)	32 (66.67%)	102 (62.96%)	134	0.639>.05 (Not Significant) (chi-square test)
Late (>3 days)	16 (33.33%)	60 (37.04%)	76	

Table 11: Association of Neonatal Profile with Neonatal Sepsis

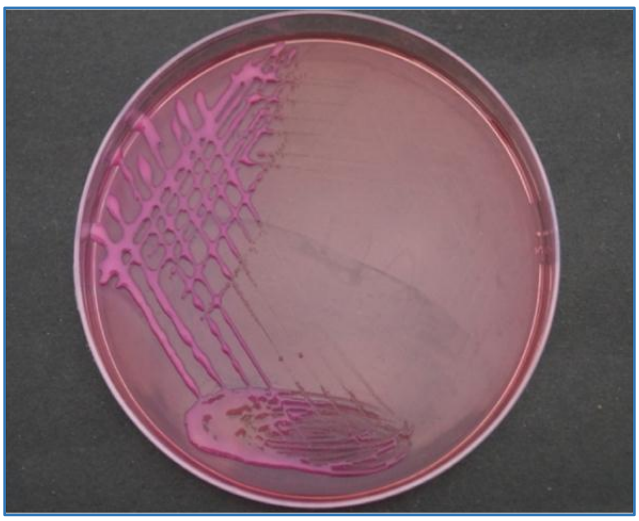

Fig. 1: Colonies of Klebsiella Pneumoniae on Culture Plate

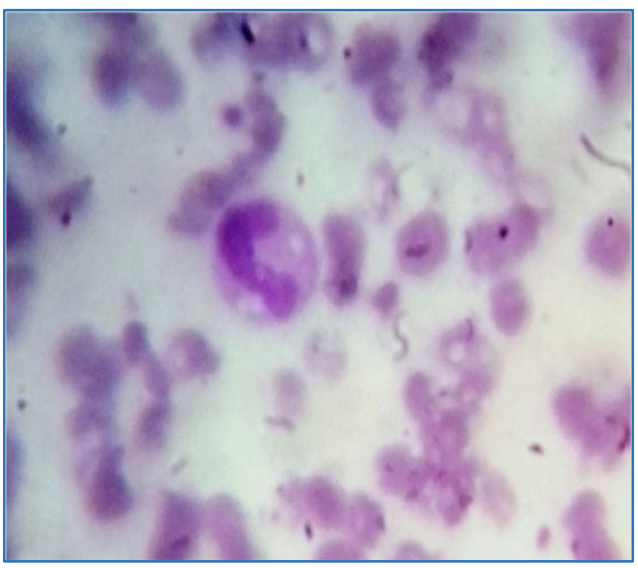

Fig. 2: Vacuolation in Neutrophils (Leishman Stain; Oil Immersion 100X)

DISCUSSION

Neonatal sepsis, sepsis neonatorum and neonatal septicaemia are terms that have been used to describe the systemic response to infection in newborn infants. The inability of neonates to completely muster the minimum inflammatory response makes them more susceptible to bacterial invasion of the blood stream than older children and adults and the risks are even higher in preterm infants. The incidence of neonatal sepsis in India is 30 per 1000 live-births (NNPD 2002-03). Early recognition and diagnosis of neonatal septicaemia is difficult, because of the variable and nonspecific clinical presentation of this condition.[4]

Neonatal sepsis is categorized as early or late onset. Eighty-five percent of newborns with early onset of infection present within 24 hours, 5% present at 24–48 hours and a smaller percentage of patients present between 48 hours and 6 days of life.[13] The susceptibility of the newborn is related to immaturity of both the cellular and humoral immune systems at birth. This feature is particularly evident in preterm neonate.

Early-onset sepsis syndrome is also associated with acquisition of micro-organisms from the mother through blood-borne transplacental infection of the foetus, ascending infection and infection upon passage through an infected birth canal or exposure to infected blood at delivery.[14] Late-onset sepsis syndrome occurs at 7-90 days of life and is acquired from the care-giving environment, prolonged hospital stay and invasive interventions.**[15]**

However, the major concern in neonatal sepsis is its non-specific presentation, sometimes the rapid progression of sepsis. Therefore, a high index of suspicion is important in the diagnosis and treatment of neonatal infection, but it is difficult to establish a diagnosis based on clinical picture alone. However, it is imperative that treatment is instituted early because of the high mortality associated with the neonatal infection. Many newborns undergo diagnostic studies and the initiation of treatment before the diagnosis has been determined. The definitive diagnosis of septicaemia is made by a positive blood culture, which requires a minimum of 48-72 h and yields a positive result in only 10-60% of cases.[8] and the facilities for the test might not be available in many laboratories.

Hence, there is a need for a reliable test for bacteraemia that is easily performed, quick, simple and cost-effective with maximum sensitivity and specificity.

In recent years, various investigators have evaluated some inflammatory markers (e.g. Procalcitonin, haptoglobins, Neutrophil CD64, interleukins, etc.) to diagnose neonatal sepsis. Also various cheap but reliable laboratory tests have been evaluated for the diagnosis of systemic infection in neonates. The Complete Blood Count (CBC) with the various neutrophil parameters and C-Reactive Protein (CRP) are the most frequently used. The present study was an attempt to assess the role of haematological parameters for the early detection of neonatal sepsis using Rodwell's Scoring System and also to find out its significance in diagnosing neonatal sepsis.

Our study showed 56.67% of neonates were males and 43.33% were female with M:F ratio of 1.3:1. The commonest age group was 24 to 48 hours (D2) with 50.95% of neonates followed by 48 to 72 hours (D3) with 23.34% and 0 to 24 hours comprised of 13.33% neonates. The mean age of the study population was 2±1 days.

The predominant maternal history included premature labour in 118 neonates followed by PROM >24 hour in 51 cases. Among the neonates, the most common presenting symptom in the early onset sepsis is respiratory distress in 91 neonates followed by tachypnoea in 74 neonates.

Out of 210 suspected cases, 48 (22.85%) neonates had sepsis by positive blood culture and 162 (77.15%) neonates were of probable sepsis (Negative blood culture). However, suspected sepsis groups (77.15%) comprises a difficult diagnostic group and could not be ignored, because fatal infection had been reported in other study in the presence of negative blood culture.[11] In the group of probable sepsis, the premature were 51.2% (83/162), whereas in the group with proven sepsis preterm neonates consisted of 72.9% (35/48) which shows a significant correlation between preterm and positive blood culture with p value of 0.007. The neonatal profile showed out of 48 cases of proven sepsis, 71% of the neonates were low birth weight <2500 grams in comparison to 29% of neonates with birth weight >2500 grams. This result was also statistically significant with p value 0.004.

This could be possibly due to impaired defense mechanisms and low immunoglobulin G levels in preterm and low birth weight neonates.[16] In addition, newborns particularly the preterm, have less effective phagocytosis and chemotactic activity. Therefore, rapid invasion of offending organism occur very fast. They also have relative immunoglobulin M deficiency rendering them more vulnerable to gram negative infections.[17]

Gram negative organisms were isolated in 30 cases (62.5%) and gram positive was found in 18 cases (37.5%). Among the gram negative organisms Klebsiella pneumoniae (18 cases) was the commonest and among the gram positive Staphylococcus aureus was the commonest organisms (7 cases), which could be compared with other studies done by Namdeo et al[18] and Mathur et al.[19]

High incidence of neonatal mortality is associated with prematurity and LBW.[20] This finding was consistent with our study. Mortality rate was 11.9%; (25/210) in our study. Out of 25 neonates 16 neonates were blood culture positive (64%), 9 neonates were blood culture negative (36%). All 16 culture positive neonates were recorded with Gram negative organisms and had a haematological score of ≥5. Out of 9 blood culture negative neonates, 3 had score ≥5 and 6 had haematological score ≥4. We observed that higher the score more chances of sepsis and vice versa, which is consistent with the study of Makkar et al.[21]

To evaluate and highlight the importance of HSS in the early detection of neonatal sepsis, we attempted different cut-offs of HSS. Out of 48 neonates with proven sepsis, all neonates had HSS ≥4 and none had score <4 ($p<0.001$). The sensitivity of HSS with cut-off score of ≥4 in predicting sepsis was 100%, specificity 69.75%, PPV 49.48% and NPV 100%. In our study, score ≥3 was also highly significant ($p<0.001$) with sensitivity of 100%, specificity of 46.91%, PPV of 35.82% and NPV of 100%. These results were similar with recent studies done by Majumdar et al[22] and Khalada et al.[8]

Haematological score ≥6 had sensitivity of 31.25%, specificity of 99.38%, PPV of 93.75% and NPV 82.99%. In comparison with haematological score ≥5, score ≥6 had higher specificity and PPV, but lower sensitivity and NPV. Considering high mortality and morbidity associated with sepsis, scores with high sensitivity and NPV are most desirable because all infants with sepsis have to be identified.[23]

In comparison with score ≥3, score ≥4 was more sensitive ($P<.001$) as Specificity and PPV were significantly higher 69.75% and 49.48% respectively. But considering the high specificity and PPV, this study implies that score ≥4 was more reliable as a screening tool for sepsis than any of the individual haematological parameter.

Among the variables of HSS it was observed that raised I:T ratio was highly sensitive in picking neonatal sepsis, while degenerative changes shown least sensitivity but higher specificity which was statistically significant ($p < 0.05$). An I:T ratio >0.2 had a sensitivity, specificity, PPV and NPV of 97.92%, 54.32%, 83.84% and 98.88% respectively. In this study, specificity and positive predictive value were low because of large number of false positive results. While an I:T ratio >0.2 suggested by Rodwell et al[11] had a sensitivity of 96% and NPV of 99%. So this result for an elevated I:T ratio

were consistent with various studies done by Manroe et al[12] and Philip et al.[24] Similar findings were also found by recent studies done by Majumdar et al[22] and Khalada et al.[8] They found I:T ratio ≥0.2 in diagnosing neonatal sepsis with sensitivity and NPV of both as 100%.

The next best indicator after I:T ratio was raised I:M ratio. The sensitivity of I:M ratio (>0.3) was 93.75%, specificity 91.36%, PPV 76.27% and NPV 98.01%. In our study, raised I:M ratio showed highest specificity. Rodwell's et al[11] used I:M ratio (>0.3) as a predictor of infection with the sensitivity of 93%, specificity 81%, PPV 32% and NPV 99%. Gosh et al[25] also found similar results.

After the raised I:T and I:M ratio, raised immature PMN count was another best single indicator of sepsis in our study. This result was similar to the observation of Gosh et al[25] and Rodwell et al.[11] Similar study was done by Khalada et al[8] in 2010. Despite a significant rise in immature neutrophil count in neonates with suspected infection, various cut-off values were examined which gave low specificity and large number of false positive result. Therefore, this parameter alone should not be evaluated for diagnostic purpose.

Neutropenia has been more common in association with sepsis compared with neutrophilia, probably because of increased adherence to altered endothelial cells and utilization at the site of infection.[26] In this study, total PMNs leucocytes count 1750-4500 cells/mm^3 (>72 hrs.) and 7800-14500 cells/mm^3 (<72 hrs.) had a sensitivity of 77.08%, specificity 38.27%, PPV 27.01% and NPV 84.93%. Similar results were observed in various studies by Rodwell et al[11], Gosh et al[25] and Zaki et al.[23] In our study, the total PMNs count was associated with low positive predictive value and low specificity. Therefore, it should not be used in isolation as a predictor of sepsis.

Total WBC count is of little clinical use in the diagnosis of neonatal infection because of wide variation in values. In this study, total WBC count had a sensitivity of 56.25%, specificity of 72.22% with PPV 37.5% and NPV 84.78%. This was in accordance with large number of studies who had observed that the total WBC count or the total PMN count show no significant association with sepsis.[27] Similar observations were also showed by Khalada et al[8] and Majumdar et al.[22] They found total WBC count in predicting sepsis had a sensitivity of 50% and 45% and NPV of 93% and 87% respectively.

Neonates with sepsis develop thrombocytopenia, possibly due to increased platelet destruction, sequestration secondary to infections, failure in platelet production due to reduced megakaryocytes or damaging effects of endotoxin.[10] In our study, thrombocytopenia ≤1,00,000/mm^3 showed sensitivity of 70.83%, specificity 59.88%, PPV 34.34% and NPV 87.39%. In our study, platelet count ≤1,50,000/mm^3 was also studied. It showed a sensitivity of 75%, specificity 31.48%, PPV 24.49% and NPV 80.95%. This parameter could be used as an early, but nonspecific marker for sepsis. Similar observations were showed in the study done by Majumdar et al[22] in 2013. They found platelet count ≤1,00,000/mm^3 in predicting sepsis had a sensitivity of 45%, NPV of 87% and specificity of 85%. Similar observations were also showed by Khalada et al.[8]

In this study, degenerative changes in neutrophils had the lowest sensitivity of 41.67%, which shows similar results with various studies by Speer et al[6], Rodwell et al[11] and Philip et al.[24] The presence of toxic granules indicates the production of unusual PMNs during infection and stress-induced leucopoiesis. They are never seen in healthy babies. Their presence indicates sepsis, but their count is not always increased (Manroe et al).[12]

Quantitative estimation of CRP was done, which was not found to have a good correlation with the neonatal sepsis. The sensitivity was only 37.5% with a specificity of 83.33%. There were conflicting results on the CRP levels in the literature.[11,22,24]

From our study, the total WBC count has little significance in diagnosing sepsis because of its wide variation in values. Total PMN count, immature PMN had low specificity and PPV. So, these should not be considered alone as a diagnostic test. Considering the morbidity and mortality, the tests having high sensitivity and NPV (I:T >0.2 and I:M >0.3) are the most desirable, as all neonatal sepsis cases should be identified. However, no single haematological test is adequate to diagnose neonatal sepsis, but when the individual haematological parameters are counted in the form of HSS, it seems to be a very effective screening method for diagnosing neonatal sepsis. Though the score ≥3 is highly sensitive, but considering the higher specificity and PPV, the score ≥4 is considered as the more reliable screening tool for the diagnosis of neonatal sepsis.

CONCLUSION

HSS increases the diagnostic accuracy of the complete blood cell count as a screening test for neonatal sepsis and simplifies and standardizes its interpretation. Though there are several methods for rapid detection of micro-organisms in blood cultures of newborn infants using automated blood culture system, DNA probe and fluorometric detection systems, still HSS can be employed as a useful test to distinguish the infected from the non-infected infants. It has high sensitivity and specificity; the certainty of sepsis being present with higher scores. However, experienced hands in evaluating blood smears are to be emphasized to minimize subjective variations associated with band count and leftward shift of myeloid immaturity measurements to improve the diagnostic yield.

Moreover, recently the light has been started throwing on the leukocyte surface marker like CD-64 quantitation in evaluating sepsis, but still in places where resources are limited the role of HSS still remains highly relevant and useful.

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