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Original Article

Infectious Disease Section

Utility of Sepsis Screen in the Early Diagnosis of Neonatal Sepsis

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ABSTRACT

Introduction: Neonatal sepsis could be defined as a clinical entity because of generalized bacterial infection within 28 days of life and showing a positive blood culture. It is probably responsible for 30-50% of the total neonatal deaths each year. Timely diagnosis of neonatal sepsis is critical because in neonates the illness can progress more rapidly than adults. The blood culture report takes at least 72 hours. Therefore, a simple test with quick availability of results can be helpful to reduce neonatal morbidity and mortality.

Aim: To evaluate the utility of sepsis screen in early diagnosis of neonatal septicemia and to study various hematological parameters, changes in peripheral blood smear, evaluate the performance of micro-erythrocyte sedimentation rate, serum C-reactive protein and serum direct bilirubin in neonates with clinical suspicion of sepsis.

Materials and Methods: The present, study was done in our institute from October 2013 to October 2015. CBC was done on 191 clinically suspected cases of neonatal sepsis along with Micro ESR, Serum CRP and direct bilirubin. Differential leukocyte count, absolute neutrophil count, immature neutrophils: total neutrophils ratio was done from Field stained peripheral smears. Blood culture was done in Microbiology Department. Exclusion criteria were neonates with major congenital anomalies and those who have already received antibiotics.

Statistical Analysis: Statistical analysis was done using SPSS software, version 20th and unpaired 't'-test.

Result: Out of 191 cases studied, 91 were culture positive. CRP (84.6%) and immature : total neutrophils ratio(75.8%) showed highest sensitivity, Whereas absolute neutrophil count(99.0%) along with serum direct bilirubin (93.0%) and corrected total leucocyte count (93.0%)showed highest specificity. Positive predictive value was highest for absolute neutrophil count (97.5%) and CRP (84.8%).

Conclusion: Serum CRP is the most sensitive marker of sepsis. Use of peripheral smear study, serum direct bilirubin and micro ESR together with CRP can be used effectively as a sepsis screen for early diagnosis of neonatal sepsis.

Keywords: Bilirubin, C - reactive protein, Leukocyte Count, Newborn

INTRODUCTION

Neonatal sepsis could be defined as a clinical entity because of generalized bacterial infection within 28 days of life and showing a positive blood culture [1]. It is probably responsible for 30-50% of the total neonatal deaths each year [1]. Neonatal sepsis can be early or late onset. Onset of symptoms within 72 hrs of life is early onset, this period can be extended up to one week. Onset of symptoms later than that is late onset sepsis. Infections are more common in low birth weight and preterm babies [2].

In neonates the illness can progress more rapidly than in adults, therefore early diagnosis is of utmost importance [2].

Positive blood culture is a gold standard for diagnosis, but it is time consuming (requires 72 hours, atleast 24 hours in case of BacT- ALERT®) and demands a well equipped laboratory [3].

Many investigators have evaluated various inflammatory markers. But these are sophisticated and impractical for developing countries [4-7].

A good diagnostic test should have high sensitivity and specificity and should be cost effective with early availability of results [4,5]. The parameters used are absolute neutrophil count, total leukocyte count, immature: mature neutrophil ratio, micro-erythrocyte sedimentation rate, C-reactive protein and serum direct bilirubin.

They together can be used as sepsis screen. Presence of two or more abnormal parameters in case of strong clinical suspicion is considered as positive sepsis screen. The results can be obtained much earlier than

blood culture and antibiotic therapy can be instituted early. This can be helpful to reduce neonatal mortality and morbidity [8].

Classification of Neonatal Sepsis: Early onset sepsis (within first 72 hours of life) and late onset sepsis (more than 72 hours) [8].

AIM

The aim of the present study was to study the various hematological parameters including various changes seen in the peripheral smears of neonates clinically suspicious of sepsis and to evaluate the performance of micro-erythrocyte sedimentation rate and C-reactive protein in neonatal sepsis.

MATERIALS AND METHODS

The present study was a cross-sectional study carried out in MGM's Medical College, Aurangabad for the duration of 2 years during period from October 2013 to October 2015 (prospective study). The study comprised of 191 neonates clinically suspected of neonatal sepsis. Ethical committee approval was obtained prior to the study.

All the neonates (age 0 to 28 days) admitted to NICU, who presented with signs and symptoms of neonatal sepsis were included in the study and those who had received the antibiotics prior to admission and neonates with major congenital anomalies were excluded from the study. Sepsis screen included following tests: Absolute Neutrophil Count (ANC), C-reactive protein (CRP), immature: total neutrophil ratio (I:T ratio), serum direct bilirubin, micro erythrocyte sedimentation rate (micro-ESR) and corrected total leukocyte count (Corrected TLC). Blood culture report was considered as a gold standard.

About 3-4 ml of blood was drawn using all aseptic precautions, out of which 1 ml of the blood sample was inoculated aseptically into a yellow BacT/ALERT pediatric blood culture bottle, 1 ml of the blood, was allowed to clot in a plane vacutainer to collect serum for the estimation of C-reactive protein and direct bilirubin. The remaining 2 ml of blood was collected in a vacutainer containing the anticoagulant EDTA (2-2.5 mg/ml) for smear preparation and estimation of the Total WBC count, Absolute neutrophil count, I: T ratio.

Total leukocyte count and absolute neutrophil count were noted from Advia 2120i automated cell counter. A drop of EDTA blood was taken on a clean dry slide and a thin tongue-shaped smear was made, air dried and stained with Field's stain. The differential count and I: T ratio was calculated as per standard hematological methods. CRP was estimated using Siemens Dimension® clinical chemistry system for quantitative determination of C-reactive protein in serum.

Micro -ESR was estimated with capillary blood obtained by heel prick, collected in a standard 75 mm micro-hematocrit tube with internal diameter of 1.1 mm. The air was not allowed to interrupt the column of blood to avoid false normal result and one end of the tube was sealed with 2-3 mm plasticin. The 45 degrees Set Square (rule) was used to draw a vertical line on the wall and the capillary tube was placed along that line. The rule was used to measure the distance from the highest point of the plasma column to the meniscus of the packed red cell column [9].

Neonatal Sepsis screen was considered positive if any two criteria of the following were present [8].

- Absolute Neutrophil Count of ≤1800/cumm
- CRP ≥1 mg/dL.
- I/T ratio ≥0.2
- Micro-ESR ≥15mm at the end of 1st hour
- Serum direct bilirubin ≥ 2mg/dL
- Total Leucocyte Count (TLC) of ≤5000/cumm

STATISTICAL ANALYSIS

All the study parameters were entered in the excel sheet and were analyzed using SPSS software, version 20. Sensitivity, specificity, positive predictive value and negative predictive value of septic screen was compared with culture outcome (gold standard). Unpaired t test was used to find p-value ($p < 0.05$ was considered significant).

RESULTS

Total 191 cases were studied in the present study. None of the babies were excluded from the study. Out of 191 cases, 91 (47.47%) cases were blood culture positive, remaining 100 (52.3%) were reported as sterile. The distribution of cases among culture positive group was as shown in [Table/ Fig-1].

Sex	Male (%)	Female (%)
	54 (59.3)	**37 (40.7)**
Birth Weight	<2.5 kg	≥2.5 kg
	74 (81.3)	17 (18.7)
Gestational Age	<37 weeks	≥37 weeks
	60 (65.9)	31 (34.1)
Age of Onset	≤3 days	>3 days
	58 (63.7)	33(36.3)
Place of Delivery	Inside NICU	Outside
	48 (52.7)	43 (47.3)

[Table/Fig-1]: The distribution of cases in culture positive group.

The percentage of male babies was more as compared to female in culture proven septicemia cases. The percentage of septicemia among low birth weight babies was considerably more than in normal birth weight babies. Preterm babies were more affected than the term babies. Early onset sepsis episodes were more than late onset. The babies born outside were affected more than the babies born inside NICU [Table/ Fig-2].

No	Parameters	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
1	Absolute neutrophil count	42.9	99.0	97.5	65.6
2	C reactive protein	84.6	78.0	77.8	84.8
3	I/T ratio	75.8	66.4	65.0	74.7
4	Micro-ESR	69.2	87.0	82.9	75.7
5	Serum direct bilirubin	57.1	93.0	88.1	79.5
6	Corrected total leukocyte count	38.5	93.0	83.3	62.4
7	Sepsis screen (two or more abnormal parameters)	93.4	77.0	78.7	92.8

[Table/Fig-2]: The predictive accuracy of the sepsis screen parameters and sepsis screen.

C-reactive protein showed highest sensitivity and negative predictive value. Absolute neutrophil count showed highest specificity and positive predictive value [Table/Fig-3]. All the parameters showed a relation with blood culture report which was statistically very significant ($p \leq 0.001$). The sensitivity, specificity, positive predictive value and negative predictive value were high for sepsis screen. The sensitivity of two or more abnormal parameters was more than any other single parameter used for screening [Table/Fig-3].

The commonest organisms were *Klebsiella pneumonia* and *Staphylococcus aureus*. Gram positive organisms were found more commonly than gram negative [Table/Fig-4].

A	Positive Blood Culture {n=91} Mean±SD	Negative Blood Culture {n=100} Mean±SD	p-value
ANC	3871.57± 3959.23	6308.54± 4176.49	p= 0.0003
CRP	3.14±3.39	0.72±0.94	p≤0.0001
I :T Ratio	0.22±0.056	0.40±2.28	p= 0.0002
Micro-ESR	17.98±5.22	11.17±3.31	p= 0.0006
S. direct bilirubin	2.33±2.03	0.73±0.71	p≤0.0001
Corrected TLC	7660.19± 4939.31	11545.63± 4982.16	p= 0.0002

[Table/Fig-3]: Comparison of mean of different parameters in positive & negative blood culture.

Micro-organisms found in blood culture	No. of Patients	Percentage
Klebsiella pneumonaie	29	31.9%
Staphylococcus aureus	26	28.7%
Candida	15	16.5%
Enterococcus species	06	6.7%
Coagulase negative Staphylococcus	04	4.4%
Escherichia coli	04	4.4%
Micrococcus	04	4.4%
Methicillin resistant Staphylococcus aureus	01	1.0%
Pseudomonas aeruginosa	01	1.0%
Streptococcus species	01	1.0%
Total	91	100%

[Table/Fig-4]: The micro- organisms detected in the blood culture report.

DISCUSSION

Definitive diagnosis rests on a positive blood culture, to identify the pathogen and determine its antibiotic susceptibility pattern, but for better survival and outcome, simple and rapid diagnostics tests are required as adjuncts to the blood culture for early and effective initiation of treatment to the septicemia neonates.

The ratio of culture positive neonatal septicemia cases were higher among males than the females in the present study, showing a ratio of 1.46 : 1. The male preponderance in neonatal septicemia may be linked to the X- linked immune-regulatory gene factor resulting in the host's susceptibility to infections in males [4] [Table/ Fig-5].

Maximum culture positive cases were seen in neonates of age ≤72 hours as compared to neonates aged more than 72 hours. The higher proportion of early onset sepsis cases may be due to the immature immunological responses of the neonates in the first week of life, making them more susceptible to infections in this period [Table/ Fig-5].

In present study, the percentage of culture positive cases in low birth weight neonates was considerably higher than in normal birth weight neonates. According to Barbara Stoll et al., [14] 1991, the rate of infection is inversely proportional to the birth weight, and low IgG levels due to impaired cellular immunity in the very low birth weight neonates contributes to the increased susceptibility to infections in these neonates [14] [Table/ Fig-6].

The sepsis was more common in preterm neonates than in term babies. Preterm babies are more susceptible to infections due to inherent deficiencies of both humoral and cellular defense mechanisms. According to Barbara J. Stoll et al., [14] the incidence of septicemia increased with the decreased gestational age of the neonates [14] [Table/ Fig-6].

In present study, the percentage of culture positive cases in neonates born inside our institute (40.68%) was lesser than the neonates born outside (50.9%). The predominant organism found in blood culture was *Klebsiella pneumoniae*, followed by *Staphylococcus aureus*. In this study, gram positive organisms

Distribution of cases according to sex						
S. No.	Authors	Year	No of cases studied (n)	Culture positive cases	Male(%)	Female(%)
1	Bhat et al., [5]	2010	1291	212	51.88	48.12
2	Sriram et al., [10]	2011	115	58	66.1	33.9
3	Swarnakar et al., [6]	2012	72	37	58	42
4	Vinay et al., [11]	2015	60	48	66.6	33.4
5	Present study	2015	191	91	59.34	40.66
Distribution of cases according to age of onset						
S. No.		Year	No of cases studied (n)	Culture positive cases	EOS (≤72 hrs) (%)	LOS (>72 hrs) (%)
1	Chacko et al., [12]	2005	36	69	55.4	44.6
2	Sriram et al., [10]	2011	115	58	77.6	22.4
3	Swarnakar et al., [6]	2012	72	37	38.09	61.91
4	Bangi et al., [13]	2014	754	120	42.62	57.38
5	Vinay et al., [11]	2015	60	48	90.0	10.0
6	Present study	2015	191	91	64.83	35.17

[Table/Fig-5]: Comparison of the studies according to sex and age of onset.

Distribution of cases according to birth weight						
No.	Authors	Year	No. of cases studied (n)	Culture positive cases	LBW (<2.5 kgs)(%)	NORMAL BIRTH WEIGHT (≥2.5 kgs) (%)
1	Sriram et al., [10]	2011	115	58	74.14	25.86
2	Mondal et al., [15]	2012	62	38	84.0	16.0
3	Pal et al., [16]	2013	238	93	71.11	28.89
4	Vinay et al., [11]	2015	60	48	70.0	30.0
5	Present study	2015	191	91	81.32	18.68
Distribution of cases according to gestational age						
No.		Year	No. of cases studied (n)	No of culture positive cases	Preterm (<37 wks) (%)	Term(≥37 wks) (%)
1	Shirazi et al., [17]	2010	138	48	69.0	31.0
2	Bhat et al., [5]	2010	1291	212	33.9	66.03
3	Khair et al., [18]	2010	100	100	91.6	8.33
4	Buch et al., [4]	2011	120	65	88.3	11.7
5	Mondal et al., [15]	2012	62	38	37.0	63.0
6	Makkar et al., [19]	2013	110	42	85.71	14.29
7	Present study	2015	191	91	65.94	34.06

[Table/Fig-6]: Comparison of the studies according to birth weight and gestational age.

(62.6%) were more commonly found in blood culture as compared to gram negative organisms (37.4%) [4-6,10,11].

Cut off value of absolute neutrophil count≤1800/µl was taken as diagnostic criterion for sepsis screen. Absolute neutrophil count in the sepsis screen showed low sensitivity (42.9%) and high specificity (99.0%). The positive predictive value was 97.5% and negative predictive value was 65.6% Absolute neutrophil count showed highest specificity and positive predictive value among all the other parameters of sepsis screen [Table/Fig-7].

C-reactive protein ≥1mg/dl was considered as positive result for sepsis screen. In present study, CRP had a high sensitivity (84.62%), specificity (78.0%), positive predictive value (77.78%) and negative predictive value (84.78%) CRP proved to be the most sensitive of all the markers of sepsis. The highest negative predictive value was seen with CRP [Table/Fig-7].

In present study, immature to total neutrophils ratio≥0.2 was diagnostic criterion for sepsis screen which had good sensitivity (75.82%), specificity (66.35%), positive predictive value (65.2%) and negative predictive value (74.71%) [Table/Fig-8].

Micro ESR≥15 mm at the end of 1 hour was considered as positive for sepsis screen. The results in the present

Predictive accuracy of absolute neutrophil count						
No.	Authors	Year	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
1	Buch et al., [4]	2010	66.15	90.91	89.58	69.44
2	Shirazi et al., [17]	2010	35.0	74.0	--	--
3	Sriram et al., [10]	2011	63.6	51.0	12.1	93.0
4	Swarnakar et al., [6]	2012	50.0	48.23	2.2	97.0
5	Jadhave et al., [7]	2013	20.0	87.5	75	36.8
6	Present study	2015	42.86	99.0	97.5	65.56
Predictive accuracy of C-reactive protein						
No.	Authors	Year	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
1	Buch et al., [4]	2010	68.466	73.64	71.83	71.43
2	Swarnakar et al., [6]	2012	52.3	56	89	14.3
3	Pal et al., [13]	2013	83.33	91.89	86.21	90.07
4	Jadhav et al., [7]	2013	90.7	37.5	73.1	68.2
5	Vinay et al., [11]	2015	81.2	50.0	86.6	40.0
6	Chacha et al., [20]	2014	62.9	73.3	37.5	88.6
7	Present study	2015	84.62	78.00	77.78	84.78

[Table/Fig-7]: Comparison of predictive accuracy of absolute neutrophil count and c-reactive protein.

Predictive accuracy of immature: total neutrophil ratio						
No.	Authors	Year	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
1	Ghosh et al., [21]	2001	93.0	95.0	92.0	90.0
2	Buch et al., [4]	2010	89.23	70.91	78.38	84.78
3	Swarnakar et al., [6]	2012	52.63	53.3	81.0	22.8
4	Jadhav et al., [7]	2013	80.0	65.0	81.1	63.4
5	Present study	2015	75.82	66.35	65.2	74.71
Predictive accuracy of micro-ESR						
No.	Authors	Year	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
1	Buch et al., [4]	2010	63.08	72.73	73.21	62.5
2	Swarnakar et al., [6]	2012	73.0	54.0	29.7	88.5
3	West et al., [22]	2012	75.7	48.1	51.0	73.5
4	Mondal et al., [15]	2012	63.0	94.0	92.0	--
5	Vinay BS et al., [11]	2015	43.0	75.0	87.0	25.0
6	Present study	2015	69.23	87.0	82.89	75.65

[Table/Fig-8]: Comparison of the studies according to immature to total neutrophil ratio and micro-ESR.

study showed specificity (87.0%) more than sensitivity (69.23%) and positive predictive value (82.89%) more than negative predictive value (75.65%). Micro ESR was a good predictor of neonatal sepsis but had lower sensitivity but higher specificity than C-reactive protein [Table/Fig-8]. Serum direct bilirubin level ≥2 mg/dl was taken as positive diagnostic criterion for sepsis screen. The sensitivity was 57.14%, specificity was 93.0%, negative predictive value was 88.14% and negative predictive value was 79.45%.Therefore serum direct bilirubin proved to be a reliable marker of neonatal sepsis. Though the sensitivity was not high but specificity, positive predictive value and negative predictive value as well were high.

Corrected total leukocyte count ≤5000/µl was taken as positive for sepsis screen. The specificity was 93.0% whereas positive predictive value and negative predictive value were 83.33% and 62.42% respectively. Leucopenia had low sensitivity, but high specificity [Table/Fig-9].

Two or more abnormal parameters had a high accuracy in predicting neonatal sepsis. The results in the present study were in accordance with Gerdes et al., [23], and Jadhav et al., [7]. The sensitivity of two or more abnormal parameters was 93.4%, specificity was 77.00%, positive predictive value was 78.7% and negative predictive value was 92.77% [Table/Fig-10]. The sepsis screen should be considered as a positive

No.	Authors	Year	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
1	Ghosh et al., [21]	2001	77.0	86.0	90.0	69.0
2	Gerdes et al., [25]	2004	100.0	83.0	27.	100.0
3	Buch et al., [4]	2010	50.77	63.64	62.26	52.24
4	Swarnakar et al., [10]	2012	66.6	50.0	10.8	94.0
5	Sriram et al., [10]	2011	63.64	50.96	12.7	92.98
6	Vinay BS et al., [11]	2015	58.0	--	87.0	28.0
7	Present study	2015	38.46	93.0	83.33	62.42

[Table/Fig-9]: Comparison of the studies according to total leukocyte count.

No.	Authors	Year	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
1	Gerdes et al., [23]	2004	100.0%	83.00%	27.00%	100.0%
2	Sriram et al., [10]	2011	55.30%	91.70%	98.30%	19.30%
3	Swarnakar et al., [6]	2012	56.0%	87.50%	97.00%	20.00%
4	Jadhav et al., [7]	2013	100.0%	62.50%	63.30%	100.0%
5	Vinay et al., [11]	2015	77%	41%	84%	31%
6	Present study	2015	93.4%	77.00%	78.70%	92.77%

[Table/Fig-10]: Comparison of the studies according to two or mor abnormal parameters of sepsis screen.

septic screen, If two parameters are abnormal and antibiotic therapy can be started. If there is strong clinical suspicion and sepsis screen is negative, in 12 hours the screen can be repeated. If the screen is negative even after that, then sepsis may not be present.

LIMITATIONS

The results obtained from sepsis screen cannot establish or rule out neonatal sepsis completely. The final diagnosis is obtained by culture and sensitivity only. The sepsis screen cannot replace blood culture. False positive cases may receive unnecessary antibiotic therapy. Obtaining blood sample in neonates is difficult.

CONCLUSION

Serum C-reactive protein is the most sensitive marker of sepsis. The presence of two or more abnormal parameters has more sensitivity than any single abnormal parameter. Use of peripheral smear study, serum direct bilirubin and micro ESR together with CRP can be used effectively as a sepsis screen for early diagnosis of neonatal sepsis. The parameters used in this study are simple, quick and cost effective. This can be useful to reduce neonatal morbidity and mortality.

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