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Rapid diagnosis of neonatal sepsis in pediatric population in tertiary care hospital, Durg

Shiv Kumar Chandraker[1], Smita Bawankar[2], Animesh Panda[3], Dhruba Hari Chandi[4]

[1]Associate Professor of Pathology, CCM Medical College & Hospital, Durg, [2]Assistant Professor of Microbiology, CCM Medical College & Hospital, Durg, [3]Assistant Professor of Microbiology, CCM Medical College & Hospital,Durg, [4]Demonstrator of Microbiology, CCM Medical College & Hospital, Durg

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ABSTRACT

Background: In under developing country Septicemia in infants to be of common occurrence with high morbidity & mortality. **Aims & Objectives:** Detection of rapid method for diagnosis of sepsis in paediatric age groups. **Materials & Methods:** Clinically suspected 369 cases of Bacteraemia in neonates, infants & children admitted as inpatients at CCM Medical College & Hospital, Durg & 45 healthy children as control were included in the present study. The cases were investigated by blood culture & 5 rapid tests Viz total leucocyte count (TLC), immature to total neutrophil (I: T) ratio, C – reactive protein (CRP), ESR & Grams smears of Buffy coat for organisms. **Results:** Blood cultures were positive in 171 (46.34%) of 369 cases and negative organisms was 55.55% as against 44.44% of Gram positive bacteria. The most common isolates were *Staph epidermides* (24.56%) and *Staph aureus* (16.37%) with overall staphylococcal prevalence of 40.93% followed by gram negative bacteria, *S.typhi* (14.61%) *E.coli* 11.11% & *Ps.auroginosa* 10.52%. **Conclusion:** The rapid tests were evaluated in blood culture positive & negative cases CRP yielded maximum sensitivity of 70.76%, Specificity of 76.26% & positive predictive accuracy of 72.02%. Combination of 2 tests did not reveal any significant advantage over single CRP test.

Key words: Bacteraemia, Blood cultures, Rapid diagnostic test & CCMMCH, Durg

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INTRODUCTION

In under developed and developing countries like India infectious diseases in infants and children are often continued to be of common occurrence with high morbidity and mortality.[1] Clinically bacteraemia is spread by transplacentally after maternal infection and invasion of the bloodstream is the usual route by which the foetus becomes infected, often difficult to diagnose due to presenting non specific clinical features with no noticeable focus of infection.[2] Since blood cultures are difficult in neonates, infants and very young children and usually require 2-3 days for diagnosis, some rapid tests have been implemented for early diagnosis of bacteraemia[3] to facilitate prompt treatment.

MATERIALS AND METHODS

The clinical material on which this study is based, were obtained from CCM Medical College & Hospital which comprised of 369 clinically suspected cases of bacteriaemia in children with 45 healthy children serving as controls.

Blood samples were collected aseptically for different tests depending upon the age of the child. About 3 - 5 ml blood was drawn from children of age 6 months and above. One ml of blood was put into a bottle containing 2 mg/ml EDTA as anticoagulant for TLC, I: T neutrophil ratio and buffy coat. One ml was allowed to clot in a sterile bijou bottle for CRP and 0.5 ml was collected in a sterile small test tube with 0.2 ml 3.8% citrate solution for ESR. Remaining 2.3 ml blood was inoculated into 20 ml Trypticase soya broth (TSB) containing 0.05% liquid in McCartney bottle. In neonates and infants finger prick blood was used for TLC, I:T ratio & at least 1ml blood was collected and clotted in a bottle for CRP and clot culture in 10 ml TSB.

The samples were immediately processed in the laboratory. Blood cultures & clot cultures were incubated at 37°C for

Address for Correspondence:
Dr. Smita Bawankar, Assistant Professor of Microbiology, CCM Medical College & Hospital, Durg, (C.G.).
E-mail: drsmita28@gmail.com. **Phone:** +8818818391.

10 days with subcultures at 3 days intervals on blood agar & MacConkey agar. The bacterial isolates were identified by biochemical reactions & special tests.[4] TLC was made using improved Neubaur counting chamber & WBC pipette and I:T ratio in Leishman's blood smears by making differential count of 100 successive neutrophils to determine nuclear indices according number of lobes of nuclei. Gram's smears of buffy coat obtained by centrifugation of EDTA blood in Wintrobe tube at 2500 rpm were examined microscopically for organisms. CRP estimation was done by latex agglutination using reagents obtained from Tulip Diagnostics limited.

The cut off values for positive tests were TLC less than 5000 and more than 20000/cmm; I: T neutrophil ratio 0.2 and above; ESR more than 10mm of first hour; CRP more than 6 mg/ml. The results of all the rapid tests were analyzed singly or in combination of 2 to assess their sensitivity, specificity and positive predictive accuracy.

RESULTS

Out of 369 cultures which includes blood and clot cultures, 171 (46.34%) yielded growth and all blood cultures were negative in controls (Table 1). All positive blood cultures revealed only monobacterial isolates. Out of 171 cultures, positive 95 (55.55%) showed growth of Gram negative bacteria and 76 (44.44%) yielded Gram positive organisms.

It is also evident from Table 2 that the most common isolates were *Staphylococcus epidermidies* (24.56%) and *Staphylococcus aureus* (16.37%) giving overall Staphylococcal prevalence of 40.93% followed by Gram negative bacteria which includes *S.Typhi* (14.61%), *E.coli* (11.11%) and *Ps.auroginosa* 10.52%.

Table 3 shows comparison between culture positive & culture negative isolates which are collected from Blood culture out of 369 clinical patients.

Table 4 shows the result of rapid diagnostic tests in 171 positive culture and 198 negative cultures cases. Gram smears of buffy coat were negative for organisms in both positive and negative cultures cases. But out of 171 blood culture positive cases, abnormal values of TLC in 98 (57.30%),I: T ratio in 89 (52.04%), ESR in 91 (53.21%) and CRP in 121 (70.76%) were observed. On the other hand, out of 198 negative culture cases, abnormal values of TLC in 86 (43.43%), I: T ratio 104 (52.52%), ESR 56 (28.28%) and in 151 (76.26%) were noticed.

Table 5 shows the results of sensitivity, specificity and positive predictive accuracy of 4 rapid diagnostic tests either singly or in combination of 2 tests were recorded in Table 3. It is evident that CRP revealed sensitivity of 70.76%, specificity of 76.26% and positive predictive accuracy of 72.02%, when compared with other tests either alone or in combination of 2 tests.

Table 1: Number and percentage of different group organisms out of 171 positive blood cultures

Organisms	Number	Percentage
Gram positive isolates	76	44.44
Gram negative isolates	95	55.55
Total	171	100

Table 2: Number and percentage of different types of organisms out of 171 positive blood cultures

Organisms	Number	Percentage
Staphylococcus epidermidies	42	24.56
Staphylococcus aureus	28	16.37
Candida albicans	06	03.50
Salmonella typhi	25	14.61
Escherichia coli	19	11.11
Pseudomonas aeruginosa	18	10.52
Proteus mirabilis	14	08.18
Klebsiella aerogenes	12	07.01
Citrobacter freundii	07	04.09
Total	171	100

Table 3: Number and percentage of culture positive and negative organisms out of 369 clinically suspected inpatients

Blood culture	Number	Percentage
Culture positive	171	46.34
Culture negative	198	53.65
Total	369	100

DISCUSSION

Bacteraemia in paediatric cases seen in hospital and clinics is of frequent occurrence with serious sequelae. Salient clinical features and some rapid laboratory tests often help to make early diagnosis.[5]

The overall smear rate of blood cultures in the present study is 46.34%. The reported positive blood cultures by different Indian authors were 50.0%,[6] 60.0%,[7] 59.9%[8] and 32.0%.[9]

Our study revealed prevalence of Gram negative bacteria as high as 55.55% when compared to prevalence of Gram positive organisms 44.44%.Further more, we observed Staphylococcal predominance of 40.93% with prevalence of *Staphylococcus epidermidies* and *Staphylococcus aureus* being 24.56% and 16.37% followed by *S.typhi* (14.61%), *E.coli* (11.11) and *Ps. aeruginosa* (10.52%). Moreover our study reported 4.09% isolation rate of *Citrobacter freundii.*

Table 4: Result of rapid diagnostic tests

Test	Positive (A)	(%)	Negative (C)	(%)	Negative (B)	(%)	Positive (D)	(%)
TLC	98	57.30	73	42.69	112	56.56	86	43.43
I:T neutrophil ratio	89	52.04	82	47.95	94	47.47	104	52.52
ESR	91	53.21	80	46.78	142	71.71	56	28.28
CRP	121	70.76	50	29.23	47	23.23	151	76.26
Buffy coat smear	0	0	0	0	0	0	0	0

(A) True Positive, (B) True Negative, (C) False Negative, (D) False Positive

Table 5: Percentage sensitivity, specificity and positive predictive accuracy of four rapid tests

Test	Sensitivity (A×100/A+C)	Specificity (D×100/B+D)	Positive predictive accuracy (A×100/A+B)
TLC	57.30	43.43	46.66
I:T ratio	52.05	52.52	48.64
ESR	53.22	28.28	39.05
CRP	70.76	76.26	72.02
CRP+TLC	64.03	59.84	59.51
CRP+I:T ratio	61.40	64.39	59.82
CRP+ESR	61.99	52.01	52.86

Out of 5 rapid tests, Gram smears of buffy coat were significantly negative in all cases. CRP showed maximum sensitivity of 70.76%, specificity of 76.26% and positive predictive accuracy of 72.02% and these findings are in agreement with the reports of other authors.[10,11] On the other hand other rapid tests singly or in combinations of 2 did not show any advantage when compared to CRP test alone. Based on our observations, we are of the opinion that blood cultures and a battery of rapid tests could be carried out depending upon the amount of blood drawn from children of different ages groups. And if blood drawn is around 1ml only CRP test could be preferred since it is a sensitive indicator of bacteraemia in the absence of blood cultures. Clot cultures could be done whenever possible.

CONCLUSION

Under the observation of neonatal sepsis, out of 5 rapid tests, CRP is the most important rapid test for the diagnosis of neonatal sepsis.

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Authors Contribution:
SKC, AP & SB: Conceptualized study, literature search, statistically analyzed and interpreted, prepared first draft of manuscript and critical revision of the manuscript. DHC: Concept of study, collected data and review of study.

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