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Original article

Rapid diagnosis of neonatal septicemia by buffy coat smear examination and C- reactive protein test in correlation with blood culture

Rajendra Prasad BPM[*a], Basavaraj KN [a], Beena Antony [b]

[a] **Associate Professor of Microbiology, J.J.M. Medical College, Davangere – 577 004 Karnataka,India.*
[b] *Professor of Microbiology, Fr. Muller Medical College, Mangalore-575002Karnataka, India.*

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ABSTRACT

Objective: To isolate the organism responsible for neonatal septicemia from blood and to correlate the blood culture results with rapid diagnostic methods like buffy coat smear examination (BCS) and C-reactive protein (CRP) test. Methods: Study was done over a period of 2 years at a tertiary care hospital in a rural set up in Karnataka State, South India. Blood samples from 200 clinically suspected neonatal septicemia cases were subjected to aerobic culture and rapid diagnostic methods like buffy coat smear examination by Gram's stain and C-reactive protein test. The blood culture results were correlated with these rapid methods. Results : Of the 200 cases studied, 95 (47.5%) were blood culture positive. Gram negative isolates were 70.53% and Gram positive 29.47%. Enterobacter cloacae and Staphylococcus aureus were the commonest organisms isolated (20% and 11.58% of cases respectively). Buffy coat had specificity of 86.1% and sensitivity of 55.3%. CRP test had 87.37% sensitivity and 71.43% specificity . Conclusion: Rapid diagnostic methods like buffy coat smear examination and C-reactive protein test needs to be evaluated with other sepsis screening methods. Blood culture remains the gold standard for the diagnosis of neonatal septicemia . Rapid diagnostic methods certainly helps in early diagnosis but has to be correlated with blood culture report.

1. Introduction

Neonatal septicemia refers to generalized bacterial infection of neonate, which includes septicemia, pneumonia and meningitis. In developing countries one of leading factors for neonatal morbidity and mortality is bacterial sepsis[1].Bacterial infections are the commonest cause of morbidity and mortality during the neonatal period. Fulminant and fatal course of infection may result from complications such as shock , disseminated intravascular coagulation and multi-system organ failure, mandating early diagnosis of this life threatening condition for a timely treatment and favorable outcome[2,3].

In developing countries ,sepsis is the commonest cause of mortality responsible for 30-50% of the 5 million total neonatal deaths each year. The reported incidence of neonatal sepsis varies from 7.1 to 38 per thousand live births in Asia [4]. National Neonatal Perinatal Database (NNPD, 2002-2003) from India has reported an incidence varying from 0.1% to 4.5% . The database comprising of 18 tertiary care neonatal units across India found sepsis to be one of the commonest causes of neonatal mortality contributing to 19% of all neonatal deaths. Septicemia was the commonest category with an incidence of 23 per 1000 live births [5].

Neonatal septicemia is difficult to diagnose clinically as it presents with non specific signs and symptoms. Traditional methods like blood culture is considered as the gold standard for diagnosis of septicemia. It takes at least 48 hours to confirm diagnosis and it causes a delay which a neonate can ill afford for initiation of appropriate therapy. Hence, certain rapid diagnostic tests were developed to diagnose septicemia and initiation of appropriate therapy to prevent morbidity and mortality [6].

* Corresponding Author : Dr. Rajendra Prasad BPM
Associate Professor of Microbiology
J.J.M. Medical College
Davangere – 577 004
Karnataka, India.
Tel No : 09448656409
E-mail: bpmrprasad@gmail.com

In the present study rapid diagnostic tests like buffy coat smear examination by Gram's stain, C-reactive protein tests were evaluated in clinically suspected septicemia in the neonates in correlation with blood culture.

1.Material and methods

The present study was conducted in the department of Microbiology of our institute which is located in a rural area in the Karnataka state, South India, over a period of 2 years . Ethical clearance was taken from the institutional ethical committee. The study group comprised of 200 clinically suspected cases of septicemia in the neonatal intensive care unit. Blood was collected from the neonates for buffy coat smear examination, C-reactive protein (CRP) test and for blood culture.

1.Buffy coat smear examination by Gram's stain

Buffy coat smear examination was done by Brooks method [7].One ml of blood was inoculated into a vial containing 10 mg ethyl diamine tetra acetic acid (EDTA) .Using long sterile needle and syringe blood was transferred to sterile Wintrobe tube and centrifuged at 2500 rpm for 15 minutes. The plasma was removed and buffy coat was taken with sterile needle .Two smears were prepared using two slide techniques and stained by Gram's stain and examined under oil immersion objective.

2. C-reactive protein test (CRP) test [8]

This test was done by latex agglutination method using kit supplied by Human diagnostic company.

A. Qualitative test

40 µl of serum sample, one drop of positive serum control and one drop of negative serum control were pipetted onto separate wells of the slide. 1 drop each of CRP latex reagent was added to all samples, mixed with separate sticks, rotated for 2 minutes and read the results. Agglutination indicated CRP content of more than 6mg /ml in undiluted serum specimen.

B. Semiquantitative test

Serum was diluted with glycine Nacl buffer in dilution of 1:2, 1:4, 1:8, 1:16, 1:32 and the test was repeated with each dilution.Visible agglutination corresponds to a CRP content of 12, 24, 48, 96, 192 mg/l respectively in undiluted specimen .

3. Blood culture and antibiogram

2 samples of blood were drawn from 2 different sites by peripheral veni puncture taking all aseptic precautions. 2 samples of 1 ml blood ,were added to separate bottles of 10ml brain heart infusion broth with liquoid .Blood culture bottles were incubated at 370c overnight .Subcultures were done next day on blood agar, Mac Conkey's agar and chocolate agar. If no growth occurred on plates after 24 hours of incubation, subsequent subcultures were done on days 2nd, 3rd, 5th and 7th. Identification of the isolates was done by studying colony morphology, Gram stain, motility and biochemical tests like carbohydrate fermentation , IMViC tests, H2s production, Oxidase test and catalase test according to the standard procedures[9].

Antimicrobial susceptibility testing of isolates was done on Muller-Hinton agar by Kirby-Baurer disc diffusion method, according to Clinical Laboratories Standard Institute(CLSI) guidelines using antibiotic discs obtained from Hi-media (Pvt.

3. Results

Out of 200 cases of suspected septicemia, 95 cases were blood culture positive. Thus prevalence of neonatal septicemia in the present study was 47.5%.

Gram negative organisms were predominant with 67 isolates (70.53%)-Enterobacter cloacae 19(20%), *Klebsiella pneumoniae* 16(16.84%), *Citrobacter freundii* 11(11.58%) ,*Escherichia coli* 9(9.47%), *Citrobacter koser*i 5(5.26%), *Pseudomonas aeruginosa* 5(5.26%) and Acinetobacter 2(2.11%). 28 Gram positive organisms were isolated in the study . Staphylococcus aureus11 (11.58%), Coagulase negative Staphylococci(CoNS) 10(10.53%),Group B Streptococci 3(3.16%), *Enterococci* 3(3.16%) and *Listeria monocytogenes*1(1.05%).

Table -1 Comparison of results of blood culture and CRP test

	Total Culture Positive	Total CRP Positive	Culture Positive CRP Positive	Culture Positive CRP Negative	Culture Negative CRP Positive	Culture Negative CRP Negative
No. of cases	95	113	83	12	30	75
Percentage	47.5%	56.5%	41.5%	6%	15%	37.5%

Table 1: Among the 200 suspected neonatal sepsis, blood culture was positive in 95 cases (47.5%) and CRP test was positive in 113 cases. Both blood culture and CRP test was positive in 83 cases,30 cases were CRP test positive and culture negative, 12 cases were CRP test negative and culture positive. In 75 cases both CRP test and blood culture turned out to be negative. Overall sensitivity of CRP test is 56.5% . Sensitivity of CRP test in blood culture proven cases was 87.37% and specificity of CRP test was 71.43%. Positive predictive value 73.45% and negative predictive value 86.21%.

Table-2 Comparison of results of blood culture and buffy coat smear(BCS)

	Total Culture Positive	Total BCS Positive	Culture Positive BCS Positive	Culture Positive BCS Negative	Culture Negative BCS Positive	Culture egative BCS Negative
No. of cases	95	63	47	38	16	99
Percentage	47.5%	31.5%	23.5%	19%	8%	49.5%

Table 2: Blood culture was positive in 95 of the 200 suspected cases and buffy coat smear was positive in 63 cases. Both blood culture and buffy coat smear was positive in 47 cases.38 cases were culture positive and buffy coat smear negative,16 cases were culture negative and buffy coat smear positive, 99 cases were both

culture and buffy coat smear negative. Overall sensitivity of BCS is 31.5%. Sensitivity of BCS in blood culture proven cases was 55.3% and specificity was 86.1%. Positive predictive value 74.6% and negative predictive value 72.3%.

Table-3 Comparison of blood culture positivity, CRP positivity and buffy coat smear positivity

Bacteria isolated	No. of Blood culture Positive	No. of CRP test Positive	No. of Buffy coat smearPositive
Gram negative organisms	67	60	34
Gram positive organisms	28	23	13

Table 3: Of the 95 blood culture positives, 67 were Gram negative isolates. Out of these 67 isolates, CRP test was positive in 60 cases and buffy coat smear was positive in 34 cases. Of the 95 blood culture positives, 28 were Gram positive isolates. Out of these 28 isolates, CRP test was positives in 23 cases and buffy coat smear was positive in 13 cases.

4. Discussion

Incidence of neonatal septicemia varied from 18.8 %[10] to 64.87%[11] in various previous studies .In our study prevalence rate was 47.5% which is in accordance with earlier reports.

Earlier studies from India, the predominant gram negative organism was Klebsiella species with an isolation rate of 24.6% to 42.2% [12,13,14,15]..In our study Klebsiella was second to Enterobacter cloacae. Mahapatra A et al [16] and Antony B and Rajendra Prasad BPM[17] reported Enterobacter cloacae as a predominant pathogen in their study. The prevalence of Enterobacter cloacae in our study was 28.35%. Marina T [18] reported Staphylococcus aureus (50.6%) as predominant pathogen. Sugandi RP et al [19] reported Staphylococcus epidermidis (18.82%) as causative agent .In our study 11strains of Staphylococcus aureus (11.58%) and 10 strains of Coagulase negative Staphylococci (10.53%) were isolated.

Buffy coat smear (BCS) study is considered as one of the rapid tests available for diagnosis of neonatal septicemia, though there are variable results. Chandna A et al [20] reported 50% sensitivity and 54% specificity with 50% positive predictive accuracy for buffy coat smear in their study. Parikh M and Singh N [6] reported acridine orange stained buffy coat smear sensitivity of 68.5% and specificity of 91.9%. Anuradha DE and co-worker [10] reported sensitivity 76.5% ,specificity 91.2%, positive predictive value 89.3% and negative predictive value 80.2% .In our study sensitivity of buffy coat smear was 55.3% and specificity 86.1% with positive predictive value of 74.6% and negative predictive value of 72.3 %.

C-reactive protein an acute phase protein in inflammation has been evaluated by many workers in this regard but the lack of specificity was the main disadvantage. Parikh M and Singh N [6] reported sensitivity and specificity of CRP test as 81.4% and 75.5% respectively in culture proven cases. Chan DK and Ho-LY [21] reported sensitivity and specificity, positive and negative predictive values as 56%, 72%, 71% and 57%. Anuradha DE and co-workers [10] in 1998, reported sensitivity of 100% and specificity of 87.3%. In the present study sensitivity of CRP test in culture proven cases was 87.37% and specificity of 71.43% respectively. Positive predictive value 73.45% and negative predictive value 86.21%.

For rapid diagnosis of neonatal septicemia, combination of buffy coat smear examination and CRP test will be very useful .Though blood culture is gold standard for diagnosis of neonatal septicemia, it takes at least 48-72 hours for blood culture report. The CRP test and BCS report will be available early. Both tests are easy to perform and rapid .CRP test is highly sensitive in cases of septicemia .Buffy coat smear is cheap, easily reproducible [10],however comparative results are not promising.

In view of changing spectrum of causative agents of septicemia and their antibiotic susceptibility patterns from time to time , a positive blood culture and antibiotic susceptibility testing of the isolates are the best guide in choosing the appropriate antimicrobial therapy in treating neonatal septicemia [22].

Conflict of interest statement

We declare that we have no conflict of interest.

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5. References

[1] Freij BJ,George H, McCraken JR.Acute infections, Chapter 48 in Neonatology – Pathophysiology and management of the new born, Philadelphia, JP Lippincott Company ,4th edn.1082-1116.

[2] Sankar MJ, Agarwal R, Deorari AK, Paul VK. Sepsis in the newborn. Indian J Pediatr 2008; 75(3): 261-266.

[3] Chacko B,Sohi I. Early onset neonatal sepsis . Indian J Pediatr 2005;72(1) :23-26.

[4] Deorari AK. Neonatal sepsis : Manageable daunting issue for India. J of Neonatol 2009;23(1): 7-11.

[5] Report 2002-2003 . National Neonatal Perinatal Database Network. New Delhi .National Neonatology Forum of India, 2004.

[6] Parikh M, Singh N. Rapid diagnosis of neonatal septicemia . Indian J Med Microbiol. 1995;13: 37-40

[7] Brooks GF, Pribble AH, Beaty HN. Early diagnosis of bacteremia by buffy coat examinations. Arch Intern Med 1973; 132.

[8] Anuradha DE, Saraswathi K,Gogate A, Raghavan K.C-reactive protein and buffy coat smear in early diagnosis of childhood septicemia. Indian JPathol Microbiol 1998;41(1) :23-26.

[9] Koneman EW,Allen SD,Janda WM , Schreckenberger PC,Winn WC.Colour atlas and textbook of Diagnostic Microbiology,Philadelphia, Lippincott,5th edn . 577-629.

[10] Anuradha DE, Saraswathi K,Gogate A, Fernandes .Bacteremia in hospitalised children – A one year prospective study .Indian J Med Microbiol 1995;13(2) :72-75.

[11] Tallur SS, Kasturi AV ,Nadigr SD,Krishna BVS .Clinico – bacteriological study of neonatal septicemia in Hubli.Indian J Pediatr 2000;67(3) :169-174.

[12] Roy I, Jain A, Kumar A, Aggarwal SK. Bacteriology of neonatal septicemia in tertiary care hospital of Northern India. Indian J Med Microbiol 2002;20(3) :156-159.

[13] Mathur M, Shah H,Dixit K, Khambadkone S,Chakarapani A, Irani S. Bacteriological profile of neonatal septicemia cases. J Postgrad Med 1994; 40:18-20.

[14] Monga K, Fernandez H, Deodhar L. Changing bacteriological patterns in neonatal septicemia . Indian J Pediatr 1988;55 :947-953.

[15] Aggarwal R, Sarkar N ,Deorari AK, Paul VK .Sepsis in the newborn Indian J Pediatr 2001;68 :1143-1147.

[16] Mahapatra A, Ghosh SK, Pattnaik D,Pattnaik K,Mahanty SK.Enterobacter cloacae: a predominant pathogen in neonatal septicemia Indian J Med Microbiol 2002;20(2) :110-112.

[17] Antony B ,RajendraPrasad BPM.An outbreak of neonatal septicemia by Enterobacter cloacae.Asian Pacific J Trop Dis 2011:227-229

[18] Marina T, Padmini B, Srimathi G,Sundararajan V,Raju BA.Microbial profile of neonatal infections in Coimbatore.Indian J Pediatr 1999;66 :11- 14.

[[19] Sugandhi R,Baliaga M, Shivananda PG. Bacteriology of neonatal septicemia in rural hospital in South India.J Trop Paediatr 1993;39:230-233.

[20] Chandna A,Rao MN, Srinivas M,Shyamala A. Rapid diagnostic tests in neonatal septicemia. Indian J Pediatr 1988;55 :947- 953.

[21] Chan DK,Ho-LY. Usefulness of C-reactive protein in diagnosis of neonatal sepsis. Singapore Med J 1997;38(6):252-255.

[22] Sriram R. Correlation of blood cuture results with the sepsis score and the sepsis screen in the diagnosis of neonatal septicemia.Int J Biol Med Res. 2011;2(1):360-368.