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Original

Septicemia detection by blood buffy coat smear in primary health care centers

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Abstract

Early diagnosis of childhood septicemia can be done by simple tests like C-reactive protein (CRP) and anticoagulant added blood centrifuged buffy coat smear (BBCS) examination, where there is no well equipped hospital setting for blood culture and identification facility in remote health care centers. This study was conducted between 1st Jan. 2007 to 27th Dec. 2007 in College of Medical Sciences, Bharatpur, Nepal. In this study we have selected 150 suspected cases of childhood septicemia for screening CRP by kit method and BBCS by two slide techniques. This kit is supplied by Span Diagnostic Pvt. Ltd. (Surat, India). Out of 150 cases of childhood septicemia of age group 0-14 years, 83 had positive C- reactive protein (CRP >6ì g/ml), 70 were positive for BBCS and blood culture was positive only in 83 cases, where predominant organism being *Klebsiella* species followed by *Staphylococcus* species. CRP test showed 100.0% sensitivity and 87.30% specificity, where BBCS showed 76.5% sensitivity and 91.2% specificity. Blood culture reports are available only after 48-72 hours and this facility is available only in well equipped centers but CRP and BBCS are easy and cheap procedure to perform even in remote areas for early diagnosis of childhood septicemia.

Key words: C-Reactive protein, blood buffy coat smear, septicemia.

Introduction

Childhood septicemia is very difficult to diagnose by manual microbiological blood culture technique because of nonspecific sign and symptoms. This takes about 48-72 hours of incubation period, according to the bacterial species causing child septicemia. These serological tests are available only in well equipped laboratory and tertiary care centers, but it is very difficult to diagnose septicemia in remote areas like district hospital and primary health care centers. CRP are early proteins, which elevate in any minor or major tissue injury or complex infections including non specifically rise in childhood septicemia.[1] For BBCS anticoagulant added blood is centrifuged and buffy coat smear is prepared for microscopy, which is very easy, cheep and reproducible technique for demonstration of bacterimia in blood.[2] These procedures are easy, convenient and time saving where report could be given within an hour. Early report will definitively help the clinician to institute antibiotics immediately just after the confirmation of the septicemia, which reduces the child mortality

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and morbidity. The main aim of this study was to evaluate the efficacy of CRP & buffy coat smear in early diagnosis of septicemic child.

Materials and methods

The study was done between 1st Jan. 2007 to 27th Dec. 2007 in College of Medical Sciences, Bharatapur, Nepal. One hundered and fifty cases of child septicemia was taken in our study, out of 150 cases 51 were male (61.4%) and 32 were female (38.5%). CRP test was carried out from the patients serum, which was collected aseptically and the test was done by using latex agglutination test kit methods, which is supplied by Span Diagnostic Pvt. Ltd. (Surat, India). The test was considered positive if value was >6 ìg \ ml. BBCS examination was done by technique of Peltela H et al.[3]

Five ml of blood sample was collected by taking aseptic precautions where 1 ml of blood was mixed with 10 mg EDTA using long sterile needle, blood was kept in a Wintrobe's tube and centrifuged at 3000 rpm for 20 minutes, now plasma was taken and buffy coat was put for smear preparation by two slide techniques and it was stained by Gram's stain, then examined under oil immersion objectives. Another 4 ml of blood was inoculated in a 45 ml of Brain Heart Infusion broth (BHI) so that blood was diluted 1: 10.[4] Blood culture bottles were incubated aerobically at 37°C for 7 days then subculture were done on 2nd day, 4th day and 7th day on Blood agar and MacConkey agar. If no growth was seen in 7th day it was considered as a negative blood culture. In culture positive cases colonies were identified by standard microbiological techniques.[5]

Results

Out of 150 children clinically suspected sepsis patients, 83 were positive for bacteria in blood culture, which indicates prevalence is 55% in our study. CRP test was positive in 106 cases, where 23 were false positive. BBCS was positive in 76 cases where 70 were true positive and 06 were false positive. 17 cases positive in blood culture did not show any organisms in buffy coat examination. **Table-I:** Shows correlation of CRP test and BBCS in 150 cases of septicemia, where 83 bacteria isolated from blood culture 56 (67.47%) were Gram negative and 27 (32.5%) were Gram positive bacteria. **Table-II:** Shows total bacteria isolated from blood culture and their relation to CRP positive and BBCS positive.

Table-I: Comparative study of CRP and BBCS among 150 cases of childhood septicemia.

Test group	Sensitivity	Specificity	Positive predictive value	Negative predictive value	False positive	False negative
CRP	100%	65.67%	87.30	100%	34.33%	00.00%
BBCS	84.34%	91.04%	92.11%	82.43%	8.96%	15.66%
CRP	92.17%	78.36%	89.71%	91.22%	21.65%	7.83%

CRP = C- Reactive protein, **BBCS** = Blood buffy coat smear.

Table-II: Correlation among positive blood culture isolates, CRP test positive and BBCS positive.

Sl. No.	Bacteria isolated	Blood culture positive	CRP test positive positive	Blood buffy coat smear positive
1.	Klebsiella pneumoniae	22(26.51%)	22(26.51%)	20(28.57%)
2.	Staphylococcus aureus	18(21.69%)	18(21.69%)	16(22.86%)
3.	Escherichia coli	11(12.25%)	11(12.25%)	11(15.71%)
4.	Acinetobacter species	08(9.63%)	08(9.63%)	06(8.57%)
5.	Pseudomonas aeruginosa	07(8.43%)	07(8.43%)	07(10.00%)
6.	Streptococcus pyogenes	04(4.81%)	04(4.81%)	03(4.29%)
7.	Salmonella typhi	03(0.36%)	03(0.36%)	02(2.86%)
8.	Serratia Species	03(3.61%)	03(3.61%)	00(00.00%)
9.	Streptococcus pneumoniae	02(2.40%)	02(2.40%)	02(2.86%)
10.	Viridans streptococci	02(2.40%)	02(2.40%)	01(1.43%)
11.	Shigella dysenteriae	02(2.40%)	02(2.40%)	02(2.86%)
12.	Enterobacter species	01(1.20%)	01(1.20%)	00(00.00%)
13.	**TOTAL**	**83**	**83**	**70**

Only few culture positive cases did not show BBCS positive, where culture positive growth was mostly Klebsella species, Staphylococcus species and *Escherichia* species. all 83 culture (with aerobic bacterial growth) positive cases showed positive CRP test. C-Reactive protein is an acute phase protein found in a concentration of up to 5 ìg / ml in serum of healthy persons however during an inflammatory response and infections, the levels may increase by as much as one thousand fold, the increase in CRP level may be detected as early as 5-10 hours after tissue damage. The increase of CRP levels in serum appears to be a non specific phenomenon but the change can be used to monitor the course of certain diseases and their treatment. The detection of CRP is more sensitive and reliable indication of inflammatory responses. The present study was under taken to assess the efficiency of CRP test and BBCS in the diagnosis of childhood septicemia. CRP concentration in serum, if it is >6 ìg / ml may be specific indication of childhood septicemia.[6] So in our study we consider only >6 ìg / ml as a positive CRP test. In this study sensitivity of CRP test is 100.0% specificity 65.6% and negative predictive value 100%. Black C et al.[7] reported sensitivity and negative predicative value (98.0%) of CRP test, which is two digit lesser than our study done in CMS-TH but specificity and positive predictive value were only 78.0% and 63.0% respectively. Another researcher Chatterjee A et al.[8] gave less i.e. 59.0% and 52.0% of sensitivity and positive predicative value of CRP test. Wench & Robin RL et al.[9] were reported 55.0% sensitivity and 82.0% of positive predictive value

of CRP in childhood septicemia, which is nearly similar to our study. In our study false positive CRP test was seen in 23 cases of viral infections which also gave positive CRP test. This elevated level of CRP can usually be demonstrated in cases of acute mycordial infections, rheumatoid arthritis, bacterial and viral infections, acute rheumatic fever with or without involvement types of heart and also renal types of malignancies.[1]

Discussion

These tests were done to assess the efficacy of BBCS in child with CRP test, which is also gets slightly elevated in subclinical infections so CRP test is specific indicator of septicemia in children[6] so in our work 6 ìg \ ml is only considered as positive test. In our study, out of 83 cases of septicemia proved by blood culture, BBCS was positive in 70 cases only. This sensitivity of BBCS in our study is 84.3%, specificity 91.0% positive predictive value 92.1% and negative predictive value 82.43. Fine gold SM et al. [5] has reported 68.5% sensitivity 91.9 % specificity and positive predictive value 88.2% of BBCS specificity reported by the latter is almost similar to our study. Hence sensitivity of BBCS in our study is higher than that already reported BBCS gave false negative results in 13 out of 70 (18.5%) due to Gram negative and 03 out of 70 (4.2%) due to Gram positive bacteria, Courtesy TA et al.[10] has missed 50.0% of Gram positive bacteria and 28.8 % of Gram negative bacteria BBCS examination false positive results were seen in 5 cases, 3 showed Gram negative and 2 showed Gram positive bacteria.

Sensitivity and specificity and passive predictive valve of CRP and BBCS combined are 92.1%, 78.3% and 89.7% respectively. The report was nearly similar to the Fellip CA et al.[11] which is 88.4%, 75.8% and 75.6% respectively, specificity of both CRP and BBCS in our study are high 65.6% and 91.0% respectively. False negative of CRP is 00.0% BBCS is 15.6%. This is the strong reason why we recommend both CRP and BBCS for diagnosing childhood septicemia instead of waiting for 48-72 hours for a blood culture report; early report of CRP test and BBCS could be available within an hour. These both the tests are easy, time saving and can be perform in remote areas as well with least expenses. This CRP test is highly sensitive, but when we compare with BBCS it is easy, cheep, time saving, rapid and also can be performed in remote centers, where there is a no well equipped laboratory available. As we know mortality is very high due to septicemia especially in neonates due to immature defense mechanism, [2] so these tests will help the clinician to start antibiotic therapy as soon as possible just after receiving reports of these tests. This will obviously reduce the chance of mortality as well as morbidity in these children.

Conclusion

We can conclude that estimation of serum CRP and BBCS is simple method for diagnosis of childhood septicemia. These tests are also helpful to rule out child infection and their treatment can be started by clinician as soon as possible within one hour, which is not only in tertiary care centers but also in remote area primary health care centers, unlike conventional methods that take upto 7 days for blood culture for diagnosis of infection and septicemia.

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