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Original Research Article

Enhanced sensitivity of Diagnosis of Neonatal septicemia using blood cultures in conjunction with C - reactive protein and Buffy coat smears

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Abstract: Neonatal sepsis is a syndrome characterized by signs of infection and accompanied by bacteria in the first month of life. Neonatal septicemia is one of the most important cause of mortality and morbidity and early diagnosis facilitates early institution of appropriate therapy. Using a combination of tests, like C-reactive protein detection, acridine orange stain of the buffy coat smears in conjunction with blood cultures will greatly enhance the sensitivity and specificity of detection of neonatal septicemia. A study was undertaken to determine the bacteriological profile of neonatal septicemia and to correlate to findings of C - reactive protein estimation and Flurososcent microscopy with buffy coat smear with acridine orange with that of the isolation of the pathogen in blood cultures. A total of 100 samples were collected from the neonates with suspected sepsis and processed by blood cultures, CRP estimation and Flurososcent microscopy with buffy coat smear with acridine orange. The results were compared. Blood cultures were positive in 58 cases (58%). The commonest isolates were Klebsiella (28, 48.2%). CRP test was positive 53 (91.38%) out of 58 culture positive cases and 7 (16.67%) out of 42 culture negative cases .Out of 58 culture positive cases , acridine orange stained buffy coat smears showed positive results in 45 cases and 3(7.14%) out of 42 culture negative cases. Used together with blood cultures, all the three tests combined, showed a sensitivity of 88.33% and specificity of 93.75% with a positive predictive value of 94.44%. A combination of tests gave increased sensitivity, specificity and positive pr.edictive accuracy compared with a single test for the diagnosis of neonatal septicemia.
Keywords: *Neonatal septicemia,* buffy coat, acridine orange stain, C-reactive protein.

INDTRODUCTION

Neonatal sepsis is a syndrome characterized by signs of infection and accompanied by bacteria in the first month of life . Neonatal septicemia is one of the most important cause of mortality and morbidity among the neonates in our country [1]. Neonates are exposed to infection more frequently because of the increased use of indwelling catheters and umbilical cannulas [2]. Bacteremia in neonates is difficult to differentiate from other conditions like anemia, respiratory distress syndrome, central nerous system injury or hypoglycemia because of clinical signs and symptoms in bacteraemia are nonspecific [3].

Early diagnosis of neonatal septicemia plays a critical role in determining the outcome of the babies. A battery of tests required to indicate the presence or absence of infection include, total leucocyte count, neutrophil count, immature total neutrophil ration, acute phase proteins (like C-reactive protein, CRP and haptogloblins), Flurososcent microscopy with buffy coat smear with acridine orange along with specific indicators like isolation of the etiological agent in the blood cultures. Blood culture is a valuable tool for isolation of fhte microorganisms and determining the antibiogram. However, the blood cultures will take about a minimum of 48 hours, some times more than 5 days to yield a positive result. It is a prudent choice to use a combination of tests to diagnose the neonatal sepsis with more sensitivity[4,5]. A combination of tests will help in early diagnosis of neonatal septicemia with early institution of antibiotic therapy.

A study was undertaken to determine the bacteriological profile of neonatal septicemia and to correlate to findings of C-Reactive Protein estimation and Flurososcent microscopy with buffy coat smear with acridine orange with that of the isolation of the pathogen in blood cultures.

MATERIALS AND METHODS :

The study was conducted in the Department of Microbiology in a tertiary care hospital in Hyderabad for a period of 6 months. A total of 100 neonates with suspected sepsis were included in the study. Neonates of low birth weight of both sexes were included in the study. Neonates presenting with signs of lethargy, refusal of feeds, fever, hypothermia, vomiting, abdominal distension, diarrohea, jaundice, seizures, respiratory distress or any external evidence of infection like umbilical cord infection, any abscess on body were noted.

About 3-4 ml of intravenous blood was collected following strict aseptic precautions The skin was cleansed over femoral vein in a circle of approximately 5cm in diameter with 70% isopropyl alcohol, rubbing vigorously. Then the area was cleaned with povidone-iodine solution which was left for about 1 minute to act. Again 70% alcohol applied over the area to remove iodine as it may cause irritation in some. After collecting 3-4ml of blood, about 1ml of blood was inoculated into blood culture bottle aseptically. 1-2 ml of blood was transferred into a bottle containing EDTA (Ethylene diaminetetraacetic acid) in a concentration of 2 mg/ml as an anticoagulant. This is used for making a buffy coat smear for fluorescent staining using acridine orange and processed within an hour. The other 1 ml of blood was allowed to clot in the bottle for estimation of CRP.

Blood cultures : the BHI broth (Brain heart infusion broth) prepared by reconstituting the commercially available powder (HiMedia) and 10ml of broth was dispensed into sterile blood culture bottles with a screw cap and rubber stopper. Addition of blood in 1:10 ratio (1ml of blood to 10 ml of broth) makes the dilution adequate to nullify the antibacterial effects in the blood. The broth was incubated at 37^0C and examined daily. Subcultures were made onto Blood agar, chocolate agar and MacConkey agar plates. The chocolate agar plates were incubated aerobically in 5% CO2 in candle jar; Blood agar and MacConkey agar plates were incubated aerobically. If there is growth, the growth is processed; if no growth, the subcultures were repeated on 2^{nd} , 3^{rd} and 7^{th} day. Antibiotic sensitivity was performed using Kirby Baeur disk diffusion method on all the isolates after identification according to standard method [6,7].

C-reactive protein assay was performed using the commercially available latex agglutination kits (Omega diagnostics, UK), with a detection limit of 6mg/dl. Any positive sample is tested in doubling dilutions for semiquantitative estimation.

Acridine Orange staining :Acridine orange is a vital stain that intercalates with nucleic acids. This binding make is fluoresce upon exposure to ultraviolet light. A stock solution of 1% acridine orange was prepared by adding 1gm of Acridine orange stain 9Loba Chemie Indo Austranel Co) to 100ml of distilled water. The stock solution when protected from light , stored at 4^0C in dark bottle is stable for 2 years. A working solution was prepared as needed by adding 0.05ml of stock solution to 0.2M acetate buffer. Smears were prepared from the blood collected into the EDTA bottles. The venous blood which was collected in the EDTA was stored at room temperature till processing, within 1 hour. The blood was centrifuged in a win trobe tube at 2500 rpm for fifteen minutes. The plasma was aspirated with a Pasteur pipette and discarded. The thin buffy layer was then aspirated along with the remaining plasma and smears were made. The slides were air dried, heat fixed and staining with acridine orange[8]. Approximately 3 minutes was required to examine each slide under fluorescent microscope. The bacteria appear bright orange, polmorphonuclear leucocytes appear yellow green. The intracellular bacteria are readily identified. The gram stain was also done on a different slide. A slide was read as negative if both smears were negative after examining 100 oil immersion fields.

RESULTS AND DISCUSSION

The study group included 100 consecutive cases of suspected neonatal septicemia over a period of 6 months, of which 58 were males and 42 were females. 38 of them were preterm infants and 72 were low birth weight. 50% of the cases were having suspected sepsis in the first week of birth (0-7 days), 32 during the second week (8-14 days) and 18 in 3^{rd} and 4^{th} weeks (15-28 days). The presenting symptoms in most of the cases were refusal of feeds, diarrohea, jaundice, lethargy and sluggish neonatal reflexes.

Blood cultures were positive in 58 cases (58%). The commonest isolates were Klebsiella (28, 48.2%), Escherichia coli (10, 17.24%), Staphylococcus aureus(4, 6.90%), Citrobacterfruendii (4, 6.90%) (Table 1).

CRP test was positive 53 (91.38%) out of 58 culture positive cases and 7 (16.67%) out of 42 culture negative cases (Table 2). Out of 58 culture positive cases , acridine orange stained buffy coat smears showed positive results in 45 cases and 3(7.14%) out of 42 culture negative cases (Table 3).

CRP alone showed sensitivity of 91.37% but a specificity of 83.33%. Acridine orange stain showed 77.53% sensitivity. But, used together with blood cultures, all the three tests combined, showed a sensitivity of 88.33% and specificity of 93.75% with a positive predictive value of 94.44% (Table 4). In a study by Anuradha De [9], the sensitivity of acridine orange stain alone was reported as 76.5% where as in

the present study it is 77.58%. various other studies have similar results ranging from 68.5% to 94.3% [8-11].

Philip et al demonstrated that a combination of tests gave increased sensitivity, specificity and positive predictive accuracy compared with a single test for the diagnosis of neonatal septicemia [12-15].

Table 1: Various isolates in blood culture

Sl No.	Name of the isolate	Number and percentage
1.	*Klebsiella aeruginosa*	28 (48.28%)
2.	*Escherichia coli*	10 (17.24%)
3.	*Staphylococcus aureus*	4 (6.90%)
4.	*Citrobacter fruendii*	4 (6.90%)
5.	*Pseudomonas aeruginosa*	3 (5.1%)
6.	*Staphylococcus epidermidis*	2 (3.5%)
7.	*Enterococcus faecalis*	2 (3.45%)
8.	*Proteus vulgaris*	2 (3.4%)
9.	*Acinetobacter sp*	1 (1.72%)
10.	*Alkaligenes faecalis*	1(1.72%)
11.	*Salmonella typhimurium*	1 (1.72%)
	Total	58

Table 2 : Results of CRP in comparison with blood cultures

CRP findings	Blood culture postive	Blood culture negative
Positive	53 (91.38%)	7 (16.67%)
Negative	5 (8.62%)	35 (83.33%)
	58	42

Table 3 : Results of Acridine orange stain in comparison with blood cultures

Acridine orange staining	Blood culture postive	Blood culture negative
Positive	45 (77.59%)	3 (7.14%)
Negative	13 (22.41%)	39 (92.86%)
	58	42

Table 4 : Sensitivity and specificity of various tests in combination

Sl No	Test/combnation	Sensitivity	Specificity	Positive predictive value	Negative predictive value
1	C-reactive protein (CRP)	91.37%	88.33%	88.33%	87.5%
2	Acridine orange stain	77.58%	93.85%	93.75%	75%
3	C-reactive protein + Acridine orange in comparision with blood cultures	88.33%	93.75%	94.44%	84.78%

CONCLUSIONS

Neonatal sepsis is a syndrome characterized by signs of infection and accompanied by bacteria in the first month of life . Neonatal septicemia is one of the most important cause of mortality and morbidity and early diagnosis facilitates early institution of appropriate therapy. Using a combination of tests, like C-reactive protein detection, acridine orange stain of the buffy coat smears in conjunction with blood cultures will greatly enhance the sensitivity and specificity of detection of neonatal septicemia.

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