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SEPTIC SCREEN AS A DIAGNOSTIC TOOL FOR NEONATAL SEPSIS : AN AUDIT

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ABSTRACT

BACKGROUND Neonatal sepsis is a leading cause of neonatal morbidity and mortality in India. Early diagnosis of neonatal sepsis is difficult due to subtle and non specific signs symptoms and symptoms . Blood culture which is the gold standard for diagnosis takes at least 48 hours and may give a false negative report particularly with prior antibiotic use. It was endeavoured to evaluate septic screen as a diagnostic marker for neonatal sepsis.

MATERIAL AND METHODS This was an observational cross sectional prospective study conducted on 500 neonates admitted to the nursery of Dr. Baba Saheb Ambedkar Hospital, with clinical features of sepsis. Following investigations were done as part of septic screen :Total Leukocytes Count,Absolute Neutrophil Count,Immature to Total neutrophil Ratio,m-ESR and C-Reactive Protein.Blood culture was done in each case.All neonates were managed as per standard ICU protocol.Septic screen was considered postive if the result of two or more parameters were positive and the result was compared with blood culture which is the gold standard for diagnosis.Data was analysed using SPSS Statistical Software Version 15.0.

RESULT In our study out of the five septic screen parameters, TLC, ANC, m-ESR and CRPwere found to be predictive of definitive blood culture positive ($P<0.05$) .CRP was the best predictor of neonatal sepsis($P<0.001$). The sensitivity of a positive septic screen was 100% while specificity was 28.6% positive predictive value of 34.3% and negative predictive value of 100% .

CONCLUSION In view of the high morbidity and mortality asociated with neonatal sepsis, septic screen with its 100% sensitivity is a useful diagnostic marker. CRP is the septic screen parameter which is most predictive of sepsis in the neonate.

KEYWORDS : Neonatal sepsis, Septic screen,diagnostic marker,CRP

INTRODUCTION

India contributes to one-fifth of global live births and more than a quarter of neonatal deaths. Nearly, 0.75 million neonates died in India in 2013, the highest for any country in the world.(1) The three major causes of neonatal deaths worldwide are infections (36%), which includes sepsis/pneumonia, tetanus and diarrhoea), pre-term (28%), and birth asphyxia (23%).(2) Sepsis is normally defined as bacteraemia in combination with systemic inflammatory response syndrome, but there is no widely accepted definition for neonatal sepsis(3) since blood culture has a low sensitivity in neonatal sepsis(4). The increased use of maternal antibiotics has reduced the rate of positive blood cultures in neonatal sepsis. In addition, bacteraemia may be transient in the early stages of disease, and the small blood volume may be insufficient to detect low bacterial density sepsis in neonates. In essence, a positive blood culture with a pathogenic organism is diagnostic of neonatal sepsis, however, a negative blood culture does not rule out the disease.

Accurate and timely diagnosis of early onset neonatal sepsis remains challenging to the clinician and the laboratory.The quest for a rapid, low cost easily available test with high sensitivity for diagnosis of neonatal sepsis continues.Various haematological parameters have been evaluated as diagnostic markers of neonatal sepsis.

In neonatal sepsis, neutropenia is a more common finding than neutrophilia, probably because of utilization at the infection site and adhesion to endothelial cells. The normal WBC count in the newborn varies but values <5000 or >20,000/cmm are abnormal.(5) A 'left shift' of neutrophils happens during sepsis because of immature neutrophils released from marrow which increases the ratio of Immature to Total neutrophils. ESR is sensitive but non-specific indicator of infection. However,ESR cannot reliably distinguish the microbial aetiology of acute inflammatory processes and takes longer time to rise after initial stimulus. Reliability of a single haematological parameter for diagnosis of neonatal sepsis is low.There are a number of factors that contribute to variability of these hematological parameters for example, maternal hypertension and perinatal asphyxia cause neutropenia. Asphyxia, maternal fever, stressful labour may elevate ITR ratio.(6)

C-reactive protein is an acute phase component which is normally undetectable but appears in serum in response to tissue injury. Previous studies have suggested that CRP is a rapid, sensitive diagnostic marker for identification of neonatal sepsis and elevation of CRP level on the background of active therapy of sepsis is a negative prognostic sign. (7,8,9) CRP is a useful marker for guiding duration of antibiotic therapy.(10)

The platelet count may fall hours to days before onset of clinical sepsis but more often remains elevated until a day or so after the infant manifests illness.

Interleukin-6 (IL-6) is an important cytokine of the early host response to infection.Its concentration increases sharply after exposure to bacterial products and precedes the increase in CRP. Some studies indicate that IL-6 is a highly sensitive marker and CRP is a more specific marker for the identification of neonatal sepsis.(11)The characteristics and kinetic propertiesof IL-8 and TNF-α are very similar to those of IL-6. Newer markers like blood lactic acid and procalcitonin are also showing promising response in the diagnosis of neonatal sepsis. (12)

Cell surface markers like Neutrophil CD11 band, CD64 are promising markers for diagnosis of early and late infections respectively. Combination of CD64, CD11b and C reactive protein further enhances the sensitivity of the expression and its negative predictive value.(13)

Diagnostic marker of neonatal sepsis should have a very high sensitivity (infected neontes test positive) and a negative predictive value (a negative test rules out infection) . The test should have a reasonably high specificity (test is negative in absence of infection) and a good positive predictive value (infection is present when test is positive), so as to limit unwarranted indiscriminate use of antibiotics in false positive cases.

However, these newer tests are expensive and not widely available in developing countries like India. So it was endevoured to use a combination of basic easily available haematological tests and find the efficacy of septic screen in early diagnosis of neonatal sepsis so that timely intervention could prevent neonatal morbidity and mortality.

MATERIAL AND METHODS

This was an observational prospective study conducted on neonates with clinical features and risk factors suggestive of sepsis presenting in the nursery of Dr. Baba Saheb Ambedkar Hospital. 500neonates were enrolled in the present study from January 2012 to December 2016.

Inclusion criteria:

Out born neonates presenting with any two or more of the following signs and symptoms were included into study:

1. Hypothermia(<950c) or fever(>990c)
2. Lethargy, poor cry, refusal to suck
3. Hypoglycaemia (<40mg/dl)/ Hyperglycaemia (>125mg/dl)
4. Poor perfusion, prolonged capillary refill time
5. Hypotonic, absent neonatal reflexes
6. Bradycardia (<100/min)/ tachycardia (>160/min)
7. Respiratory distress, apnoea and gasping respiration
8. Bulging anterior fontanel, vacant stare, high-pitched cry, excess irritability, stupor/coma, seizures, neck retraction
9. Feed intolerance, vomiting, diarrhoea, abdominal distension
10. Bleeding , petichae , purpura
11. Multiple pustules (>10), abscess, sclerema, mottling, umbilical redness and discharge.

Exclusion criteria

1. Neonate with obvious congenital malformation or TORCH infection
2. Neonate born to HIV positive mother.
3. Neonate who have received antibiotics(oral/intravenous).
4. Neonate with history of birth asphyxia(APGAR score <5 at 1 min).

The neonates enrolled into the study were classified into three categories as follows:

1. **No sepsis** neonate presenting with clinical features and risk factors suggestive of sepsis but Septic screen and blood culture were negative.
2. **Probable sepsis** neonate presenting with clinical features and risk factors suggestive of sepsis .Septic screen was positive and blood culture was negative.
3. **Definitive sepsis** neonate presenting with clinical features and risk factors suggestive of sepsis and both Septic screen and blood culture were positive.

At presentation to the Paediatric Emergency Department, the neonates were assessed and emergency resuscitative measures and treatment was given as per the NICU management protocol. Following this, a questionnaire was completed.

Following investigations were done as part of septic screen :

1. Total Leukocytes Count
2. Absolute Neutrophil Count
3. Immature to Total neutrophil Ratio
4. m-ESR
5. C-Reactive Protein

For TLC,ANC,ITR and CRP,blood samples 2.0 ml was drawn in EDTA and plain vial and peripheral blood smear was prepared for band cells/immature cells and sent to dept of pathology and microbiology for the result. For micro ESR heel prick blood was collected in graduated and pre-heparinised capillary tube and kept straight for 1 hr for results.

A sterilized, sealed pack blood culture bottle of volume 40 ml containing BACTEC Peds plus TM/F culture vial was used for blood culture. After aseptically injecting the blood, the inoculated aerobic vials were placed in the BACTEC fluorescent series instrument as soon as possible for incubation and monitoring.

Other investigations on the basis of indication:

1. Chest-X-Ray: in patient with history of respiratory distress
2. X-Ray abdomen : for necrotising enterocolitis
3. Liver function test
4. Kidney function test
5. Serum electrolyte
6. ABG
7. Ultrasonography of head
8. CSF examination etc.

While awaiting test results, based on clinical suspicion we started intravenous antibiotics. This was later modified/discontinued on the basis of test results. The duration of antibiotics depends upon the diagnosis i.e. for pyogenic meningitis (3wks), for definitive sepsis (2wks), for probable sepsis (7days) and for no sepsis (3-5 days).

Interpretation of rapid screening test for neonatal sepsis(14,15):

1. Total leucocytes count (TLC) <5000/cmmor> 20000/cmm was considered positive.
2. Absolute neutrophil count: ANC < 1800/cmm was considered positive.The lower limit for ANC in the newborn begins at 1800/cmm, rises to 7200/cmm at 12 hours of age and then declines and persists at 1800/cmm after 72 hours of age.
3. Immature (band cell) / total neutrophil ratio (ITR):ITR >20 % was considered positive in preterm baby78 and >27 % was considered positive in term baby78
4. micro- ESR:In neonate ≤ 7 days, Value > age in days + 3 mm in Ist hour was considered positive and in neonate >7 days, value >10 mm in Ist hr was considered positive.
5. C-reactive protein: >1 mg/dl was considered positive.

If any ≥ 2 of the above mentioned five parameters were found to be positive than septic screen was considered to be positive.

Statistical analysis:

Qualitative variables were summarized in terms of percentages and their significance across the categories of sepsis was evaluated using chi-square test.

Also the mean ± SD was computed for quantitative variables and their significant difference was tested by using ANOVA / Kruskal -Wallis test.

Ethical consideration:

All the study methods have been approved by the ethical committee of Dr. Baba Saheb Ambedkar Hospital, Delhi. Informed consent was obtained from the parents at the beginning of the study.

OBSERVATION AND RESULTS

In this study conducted from January 2012 onwards, 500 neonates fulfilling the inclusion criteria were enrolled in the NICU of Dr. Baba Saheb Ambedkar Hospital, Delhi.

Out of the 500 neonates admitted, 315(63%) were male and 185(37%) were female. Although male sex was predominant in sepsis with the ratio of male: female being 1.7: 1.There were 186 (37.2%) preterm and 314 (62.8%) term neonates. 248(49.6) of the neonates in the study population were low birth weight. According to the age of onset of infection, neonates were classified as EOS (≤ 3 days) and LOS (>3 days). There were 128(25.6%) neonates admitted as EOS and 372(74.4%) admitted as LOS.

All the neonates underwent septic screen which included TLC, ANC, ITR, CRP, m-ESR. If any of the ≥ 2 value out of these five were found to be positive then septic screen was considered to be positive. The septic screen was found to be positive in 396(79.2%) and negative in 104(20.8%) of cases.

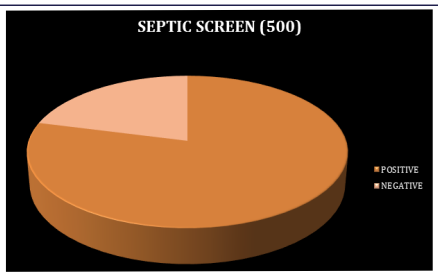

FIGURE I: PERCENTAGE OF SEPTIC SCREEN POSITIVE AND NEGATIVE CASES

TABLE II: RELATIONSHIP BETWEEN SEPTIC SCREEN AND BLOOD CULTURE POSITIVE NEONATAL SEPSIS

SEPTIC SCREEN	BLOOD CULTURE		
	POSITIVE	NEGATIVE	TOTAL
POSITIVE	136	260	396
NEGATIVE	0	52	104
TOTAL	136	364	500

The blood culture was found to be positive in 136(27.2%) cases. 136(27.2%) cases were culture positive definitive sepsis, 260 (52%) were septic screen positive but blood culture negative probable sepsis and 114(20.8%) were of no sepsis group.

The sensitivity of a positive septic screen was 100% while specificity was 28.6% positive predictive value of 34.3% and negative predictive value of 100% .

TABLE II: RELATIONSHIP BETWEEN DIFFERENT VARIABLES OF SEPTIC SCREEN AND SEPSIS

	SEPSIS			P value
	NONE (n=104)	PROBABLE (n=260)	DEFINITIVE (n=136)	
TLC (cmm)	13369.23± 3453.67	17230.77± 7811.53	20126.47± 10752.46	0.041
ANC (cmm)	5735.46 ± 1984.78	6337.11 ± 5064.72	7517.35 ± 4378.57	0.048
ITR	22.27 ± 4.01	23.51 ± 3.73	23.29 ± 4.36	0.401
m-ESR (mm in 1st hr)	9.88 ± 3.31	12.35 ± 4.64	14.35 ± 5.77	0.002
CRP (mg/dl)	0.74 ± 0.45	2.63 ± 0.94	2.89 ± 1.06	<0.001

Of the septic screen parameters, TLC, ANC, m-ESR, CRP were predictive of blood culture postive neonatal sepsis and the difference was found out to be statistically significant with p-value <0.05. ITR was not significantly associated with culture positive sepsis p-value > 0.05.

DISCUSSION

According to National Neonatal Perinatal Database (NNPD) of India (16), enrolling 151,436 intramural deliveries at 18 tertiary centres, incidence of neonatal sepsis was 3.0%. Sepsis was confirmed by blood culture in 28.6% cases of clinical sepsis. Infection was cause of mortality in 18.6% of total neonatal deaths.Similar rate of blood culture positivity was noted in the present study wherein 136(27.2%) cases were culture positive definitive sepsis,260 (52%) were septic screen positive probable sepsis and 114(20.8%) were both septic screen and blood culture negative .

Isolation of micro-organisms from one or more blood cultures is the gold standard to establish a definite diagnosis of neonatal sepsis. The sole use of blood culture to diagnose neonatal infection has a number of limitations. It may take 48 to 72 hours to obtain culture results. Although a positive blood culture is generally considered to be the gold standard for diagnosis of septicaemia, spurious results from contaminated samples are not infrequent.Also there may be negative culture report with the use of antibiotics.

The timely and accurate diagnosis of sepsis in newborns has proved to be a challenging task for the treating physicians since many years however, no dependable single tests are accessible. In this study it was endeavoured to find the reliability of a combination of five parameters(septic screen) in detection of neonatal sepsis. As no single individual haematological parameter is superior in comparison to another in predicting neonatal sepsis, a combination of these parameters in the form of septic screen has been recommended (17).

The five parameters included into the septic screen in the present study were TLC, ANC, ITR, m- ESR and CRP. Any 2 or more positive values were considered to be a marker of neonatal sepsis. In our study out of the 5 variables 3 variables i.e. TLC, ANC and m-ESR were came out to be significant with P values <0.05 while the one variable i.e. CRP came out to be highly significant with P value < 0.001.

C-reactive protein is an acute phase component which is normally undetectable but appears in serum in response to tissue injury.**In our study CRP was found to be the best predictor of definitive sepsis in comparison to the othe haematological markers.**

Stephan et al in their study on neonatal sepsis found that CRP is a useful marker for guiding duration of antibiotic therapy and the approach allowed considerably shorter course of antibiotic.(10) Jinchardze N et al concluded that CRP is a rapid, sensitive diagnostic marker for identification of early-onset sepsis in preterm infants. He also suggested that elevation of CRP level on the background of active therapy of sepsis is a negative prognostic sign.(8) In a prospective study carried out by Jumah DS and Hassan MK (9) statistically significant higher mortality was reported in neonates having thrombocytopenia, neutropenia and Creactive protein ≥10 mg / dl. Vinay BS et al found CRP the single best diagnostic test of the various indicators of sepsis. (18).When considered with any of the hematological parameter, the sensitivity, specificity, PPV and NPV reduced. Ucar B et al concluded the order of the markers according to sensitivity and specificity for optimum prediction of neonatal sepsis is CRP > PCT > TNF-α > SAA.(19)

The sensitivity of a positive septic screen in the present study was 100% while specificity was 28.6% positive predictive value of 34.3% and negative predictive value of 100% .The very high sensitivity makes it an ideal diagnostic test for neonatal sepsis which carries a high morbidity and mortality as all infected neonates are detected. The low sensitivity and positive predicted value when blood culture is taken as the gold standard for diagnosis may be explained by the fact that blood culture may be negative in the presence of maternal antibiotic use,low bacterial load in the neonate or due to the small size of inoculum in blood culture bottle. Gerdes et al in a similar study found sensitivity 100%, specificity 83% , positive and negative likelihood ratio as 5.9 and 0.00 respectively.(20) Vinay BS et al concluded that a positive septic screen had sensitivity of 77%, specificity of 41%, positive predictive value of 84% and negative predictive value of 31% when blood culture is considered as gold standard to detect neonatal sepsis. (18)Lakhey et al established that the sensitivity of two or more abnormal parameters was 90.3%, specifpity was 75.6%, positive predictive value was 77.0% and negative predictive value was 89.0% .(21)

CONCLUSION

The diagnosis of neonatal sepsis is still an uphill task for the clinician due to non-specific clinical presentation,absence of standardized cut-off values for haematological parameters, and low positivity and delay in blood culture report.

The present study established that a positive septic screen has sensitivity of 100%, specificity of 28.6%, positive predictive value of 34.3% and negative predictive value of 100% when blood culture is considered as gold standard test to diagnose neonatal sepsis.

These basic haematological tests can be easily performed in health facilities with minimum infrastructure to facilitate early detection and treatment in cases of neonatal sepsis in the periphery.

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