Original Research Article

Early onset neonatal sepsis in relation to prolonged rupture of membranes of more than 18 hours - A prospective study

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Abstract

Background: Sepsis is the commonest cause of neonatal mortality globally and is responsible for about 3–50% of the total neonatal deaths in the developing countries. In India, sepsis contributes up to 52% of neonatal deaths in the community and 36% of deaths in hospital newborns. Prolonged rupture of membranes (PROM) is a common and significant cause of early onset sepsis and preterm labour. The evaluation of neonatal sepsis is important and there is an urgent need to know whether the baby has sepsis so as to institute treatment as quickly as possible. **Objective:** To determine the number of neonates with early onset sepsis in relation to PROM of more than 18 hours. **Materials and Methods:** Prospective hospital based study conducted at Paediatric Department, KIMS Hospital, Hubli from December 2011 to November 2012. Neonates born to mothers in KIMS hospital, Hubli with history of PROM of more than 18 hours were evaluated clinically and a set of investigations in relation to sepsis done immediately. **Statistical Analysis:** Data was analyzed using SPSS software for Windows version 20.0, categorical tables, Chi-square values, Fischer extract test and the results correlated. **Results:** In newborns with PROM >18 hours, the septic screen was positive in 68 cases. Blood culture was positive in 21 neonates; the most common organism grown was Klebsiella. **Conclusion:** Culture proven sepsis was seen in 21 neonates with PROM >18 hours; the most common organism being Klebsiella. Probable sepsis was detected in 68 neonates. Longer duration of PROM was associated with higher incidence of sepsis

Key Words: Blood culture, Prolonged rupture of membranes, Sepsis.

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INTRODUCTION

Sepsis is the commonest cause of neonatal mortality globally and is responsible for about 3–50% of the total neonatal deaths in the developing countries. In India, sepsis contributes up to 52% of neonatal deaths in the community and 36% of deaths in hospital newborns[1]. In the era of advanced life supporting systems and antibiotics, neonatal sepsis remains a significant and frequent cause of morbidity and mortality, particularly in the developing countries. Neonatal sepsis is one of the most widely studied disorders in paediatrics[2]. The subtle signs of septicaemia are common to various illnesses; therefore, clinical diagnosis of neonatal sepsis is very difficult[3,4]. Isolation of micro-organisms by blood culture is the definitive diagnosis of sepsis, but this is time consuming (48 to 72 hrs) and requires a well-equipped laboratory. Considering the course of disease, early diagnosis of this particular condition is very much desired. Most paediatricians administer antibiotics to neonates at the least suspicion of clinical sepsis. This policy of empirical antibiotic usage might result, and in many cases, has resulted in the emergence of resistant organisms, which may be very difficult to treat. The most commonly used diagnostic tests for neonatal sepsis still

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are neutrophil indices and C-reactive protein (CRP). A wide variety of newer diagnostic tests (eg. cytokine determinations, receptor levels, adhesion molecules, and procalcitonin levels) have been used to identify newborn infants with possible infections. Most require several hours until results are available and are not practical for clinicians who need quick feedback[3].No diagnostic test, can, or will ever, supplant the careful physical examination and close monitoring of infants. Prolonged rupture of membranes (PROM) is a common and significant cause of early onset sepsis and preterm labour[5].In our hospital, the number of neonates with early onset sepsis is high and we have a large number of deliveries with history of PROM of more than 18 hours. Hence this study is undertaken to determine the number of neonates with early onset neonatal sepsis in relation to PROM of more than 18 hours. So with this background, we conducted a study to determine the number of neonates with early onset sepsis in relation to PROM of more than 18 hours.

MATERIALS AND METHODS

Source of Data: Neonates born to mothers with history of PROM of more than 18 hours delivered in KIMS Hospital, Hubli.

Type of the study: Prospective hospital based study.

Inclusion Criteria: Neonates born to mothers with history of PROM of more than 18 hours in KIMS Hospital, Hubli

Exclusion Criteria

1. Neonates who underwent any mode of resuscitation other than routine care.
2. Newborns delivered outside KIMS, Hubli
3. Newborns with MSAF or MAS
4. Newborns with major congenital anomalies

Method of collection of data

The study period was from 1-12-2011 to 30-11-2012.Written informed consent was taken from the parents. Neonates born to mothers in KIMS hospital, Hubli with history of PROM of more than 18 hours were included in the study. They were evaluated clinically and a set of investigations in relation to sepsis done immediately. The blood samples were collected under all aseptic precautions in NICU, in non-siliconised vaccutainer tubes with tripotassium EDTA as an anticoagulant. Another 2 ml was taken for conventional blood culture. Also 1ml blood sample was taken for estimation of semi-quantitative CRP levels.

Total leukocyte Count: Sysmex auto analyser was used to analyse the sample and counter checked. Leukopoenia with count less than 5000 cells/$mm^3$was considered as evidence of sepsis.

Differential Count: Peripheral blood smears were prepared using smooth edged glass spreader resulting in tongue shaped smears, stained with Leishman stain and examined under oil immersion light microscopy at a final magnification of 1000. Differential counts were performed on Leishman stained smears and about 300 cells were counted. Absolute neutrophil count (ANC) of less than 1750 cells/$mm^3$was considered as evidence of sepsis. Likewise, I/T neutrophil ratio of more than 0.2 was considered as a case of sepsis.

Micro- Erythrocyte Sedimentation Rate (Micro-ESR): Micro-ESR of more than 15 mm at the end of 1 hour was considered significant. It was done by using pre-heparinised micro-hematocrit tube with internal diameter of 1.1 mm and total length of 75 mm. It was filled with capillary action. Air was not allowed to interrupt the blood column. Lower end of tube was closed with plasticin and then tube was fitted vertically by means 21 of sticking plaster. The fall of erythrocyte at the end of the hour was measured accurately to the nearest millimetre.

C - reactive protein (CRP): The CRP was estimated semi-quantitatively by the latex slide agglutination method. Principle: The latex slide agglutination test is based on the immunological reaction between CRP antigen and latex particles coated with mono specific anti-human CRP antibody. The kit consisted of a plastic slide with six reaction circles, sample dispensing pipettes, mixing sticks, rubber teats, CRP latex reagent, and positive and negative controls. Serum obtained from blood collected in plain bulb was used as test sample. Method: Bring the serum samples and reagents to the room temperature. Using a plastic dropper, a drop of test serum was placed within the area on the glass slide. One drop of latex CRP reagent was added to it, taking precaution that the dropper tip does not touch the liquid on the slide. Using mixing stick provided, the serum and CRP latex reagent is mixed and spread uniformly over the entire circle. Rock the slide gently back and forth. Look for macroscopic agglutination at exactly two minutes in a direct light source. Positive and negative controls are also run simultaneously. Results were interpreted as follows: Agglutination seen macroscopically shows positive test indicative of a CRP concentration equal to or above 1 mg/dL. Smooth suspension without any noticeable agglutination shows negative test indicative of a CRP concentration below 1 mg/dL.

Blood Culture: A sample of 2cc of blood was collected in a conventional blood culture bulb using brain heart infusion as the transport media. It was incubated and grown on chocolate and Mc-Conkey media. On detection of growth of any organisms, it was reported in 3 days and was further incubated and sub cultured. Antibiotic

sensitivity was detected appropriately. A report of no growth was given when there was no positive growth in any plates after a period of one week. The cut off values for the positive rapid screening tests in this study were as follows[6]:

1. Total leukocyte count (Leucopenia) : <5,000cells/mm3
2. Absolute neutrophil count (Neutropenia) : < 1750cells/mm3
3. I/T Neutrophil ratio: > 0.2
4. Micro ESR: >15 mm in the 1st hour
5. CRP: >1mg/dl

Statistical Analysis: All the findings were recorded and comparisons drawn between clinical profile, blood culture results, and the sepsis screen tests. Data was analyzed using the SPSS software for Windows version 20.0(Statistical Presentation System Software, IBM Inc. New York) and categorical tables, Chi-square values, Fischer extract test and the results correlated. Conclusions were drawn from the tabulated results. Test result is considered significant if *p* value is less than 0.05 (i.e.5%)

OBSERVATIONS AND RESULTS

A total of 100 neonates born to mothers with PROM >18 hrs were included in the study. Among the cases, 64 and 36 were male and female babies. Majority (83) have PROM of 18-24 hrs duration. The duration of 24-48 hrs was observed in 15 and >48 hrs observed in 2 mothers.12% mothers had fever and 8% had history of passing foul smelling liquor. Vaginal delivers were predominant with 62 compared to LSCS (38).A total of 59% term and 41 preterm babies were included in the study. The no: of IUGR babies was 15.The mean birth weight was 2.221 kg (+- 0.526). The maximum weight recorded was 3.5 kg and a minimum of 950 grams. The median birth weight was 2.2 and the most commonly occurring weight was 2.5 kg. The majority of neonates (53%) showed CRP positive while it was negative in 47 babies. blood culture was positive in 21 neonates while it was negative in 79 cases. The maximum number was of Klebsiella growth observed in 30 %, followed by CONS in 25%, Pseudomonas in 20%. ECOLI growth was noticed in 15 % whereas Candida was grown in 10%.A total of 68 neonates born with PROM >18 hours had a positive septic screen where as it was negative in the rest 32 neonates. Sepsis was detected in 43/64 males (67%) and 24/36(66%) females.

Table 1: Septic Screen and Gestation

Gestation	No:	Probable Sepsis No:	%
Term	59	47	79
Preterm	41	21	51

Chi-square-3.91, Degrees of freedom = 2, Probability = 0.142

Table 14 enumerates the relationship between gestational age and probable sepsis. The septic screen was positive among 47/59 term neonates and in 21/41.

Table 2: Probable sepsis among IUGR babies

	Total No:	Probable sepsis	%
AGA	85	57	65.5
SGA	15	11	73.3

Table 2 represents the relationship between probable sepsis and IUGR babies. Among the 15 IUGR babies, 11 had features of probable sepsis, constituting 73.33%.

Table 3: Birth Weight and Probable Sepsis

Birth WT	No:	Probable Sepsis	%
Normal	44	31	70.45
LBW	49	32	65.3
VLBW	6	4	66.66
ELBW	1	1	100

The given table has probability 3.5E-02The sum of the probabilities, $p = 0.922$

Table 3 enumerates the relationship between birth weight and probable sepsis. Septic screen was positive in 70.5% of neonates with normal birth weight. Among the babies with LBW, 66.5% and 66.7% of VLBW babies had a positive septic screen. ELBW babies showed a 100% positive septic screen; not statistically significant.

Table 4: Mode of Delivery and Probable Sepsis

Mode of Delivery	No:	Probable Sepsis	%
LSCS	38	19	50
Vaginal	62	49	76.56

Table 4 differentiates the neonates with a positive septic screen according to their mode of delivery. Among those babies delivered by vaginal route, 76.6% had a positive septic screen compared to 50% in those delivered by a LSCS.

Table 5: Duration of PROM and Sepsis

PROM	No:	PROBABLE SEPSIS	%	BLOOD CULTURE POSITIVE	%
>18	83	56	67.46	14	16.86
>24	15	10	66.66	15	33.33
>48	2	2	100	2	100

Probable sepsis: probability 0.1 and $p = 1.000$, Blood culture: probability 3.9E-03 and $p = 0.013$ Table 5 enumerates the correlation between duration of PROM and incidence of probable sepsis and positive blood culture. Among those babies born with PROM >18 hrs, positive septic screen was seen in 67.5% and a positive blood culture in 17.9%. The incidence of probable sepsis was 66.7% and 33.4% positive blood culture among those with PROM duration of >24 hrs. Neonates with history of PROM >48 hrs had a 100% positive septic screen and blood culture; not statistically significant.

Table 6: Foul Smelling Liquor and Sepsis

FOUL SMELLING LIQUOR	No:	%
Total No:	8	
Sepsis	8	100
Blood culture	3	37.5

Table 6 delineates that a positive septic screen was seen in 100% of neonates born with foul smelling liquor, though the blood culture was positive in a 37.5%.

Table 7: Gestation and Blood Culture

Gestation	No:	Positive Blood Culture	%
Term	59	12	20.33
Preterm	41	9	21.95

Chi-square = 0.460E-01degrees of freedom = 2 probability = 0.977

Table 7 defines the blood culture positive cases according to gestational age of the neonates. In term neonates a 20.33% and in preterm's 21.9% had blood cultures positive; not statistically significant.

Table 8: Blood culture positivity among IUGR babies

	Total No:	Positive blood culture	%
AGA	85	18	21.17
SGA	15	3	20

Table 8 enlightens the relationship between positive blood cultures among IUGR babies. Among the 15 IUGR babies, 3 had a positive blood culture (20%).

Table 9: Birth Weight and Blood Culture

Birth Weight	No:	Positive Blood Culture	%
Normal	44	9	20.45
LBW	49	10	20.49
VLBW	6	1	16.66
ELBW	1	1	100

Probability 1.7E-02The sum of the probabilities of tables, $p = 0.416$

Table 9 enumerates the relationship between birth weight and positive blood culture. Blood culture was positive in 9/44 in normal weight, 10/49 LBW and 1/16 VLBW babies. Blood culture as positive in 100% ELBW babies; statistically significant.

DISCUSSION

Neonatal sepsis is one of the major factors contributing to the high perinatal and neonatal mortality and morbidity. Surviving infants can have significant neurologic sequelae as a consequence of central nervous system involvement, septic shock or hypoxemia secondary to severe parenchymal lung disease[7].EOS, with an onset during the first 72 hours of life, is caused by organisms prevalent in the maternal genital tract or in the labour room and maternity operation theatre. The pattern of organisms causing sepsis differs from place to place and can change in the same place over a period of time. The present study was carried out to determine the number of neonates with EOS in relation to PROM >18 hrs.

Clinical profile

Sex: In the present study, it was found that the incidence of sepsis was higher in males Wilson *et al*[8] observed male predominance because congenital anomalies of urinary tract are more in males, which gives rise to urinary tract infection. In females, resistance occurs due to their heterozygosity for gene of X-chromosome controlling immunoglobulin synthesis. Saxena *et al*[9] reported male predominance in 59.1% of cases.

Weight: Table 9 shows 20.49% of the cases were having weight less than 2.5kg, who were bacteriologically positive for neonatal sepsis. This observation was consistent with other studies. Betty C et al[6] reports that among infants with EOS, 83.3% were LBW, 30.5 % VLBW. Gupta *et al*[10] found that in 55% of cases, the weight at the time of the disease was less than 2000gm.

Maturity: Table 8 shows that in our study, EOS was present in 21% of preterm babies. Betty C *et al*[6] states the incidence of EOS in 80.6% preterm babies. Misra *et al*[11] stated higher incidence of sepsis in premature and low birth weight.

Blood Culture: In the present study Klebsiella were the most common microorganisms to be isolated (30%) followed by CONS (25%), Pseudomonas (20%). Russell G.A.B *et al*[18] have reported that staphylococcus epidermidis was the most common organism isolated. Asini *et al*[12] reported a 31% and Kite P *et al*[13] reported that 62% of the organisms isolated in their study were coagulase negative staphylococci. Betty C *et al*[6] reported that Pseudomonas (60%) was the most common organism isolated. Zawar M.P *et al*[14] reported that gram positive organisms like staphylococcus, both coagulase positive and negative were more commonly isolated. Hasan B *et al*[5], Varsha *et al*[16] and Sharma A *et al*[17] in their studies have found that Klebsiella was the most common microorganism isolated. Siegel *et al*[3] and Wilson *et al*[8] stated that the organism causing neonatal sepsis differ from place to place, country to country, and from nursery to nursery.

C - REACTIVE PROTEIN: In the present study CRP was positive in 47% of neonates.CRP may be elevated in other neonatal and obstetric conditions limiting the accuracy of the test. In a study conducted by Hassan B *et al*[9]CRP was positive in 20 neonates.

SEPSIS SCREEN: In the present study 68 neonates had a positive septic screen-when the sepsis screen score was ≥2.The components of the sepsis screens have varied from only haematological parameters, which can be available from a complete blood count, to many combination of acute phase reactants, inflammatory mediators etc, with the leukocyte indices. In this study a combination of hematologic parameters like TC, ANC, I/T ratio, Micro ESR and CRP were used to formulate a

similar sepsis screen. Hasan B *et al*[9] states a 45% neonate with probable sepsis.

CONCLUSION

Blood culture positive sepsis was present in 21 neonates born with PROM >18 hrs. Probable sepsis was present in 68%.Neonatal sepsis was more common in preterm and low birth weight babies. There was male predominance in neonatal sepsis. The most common organism detected in blood culture was Klebsiella (30%) followed by CONS (25%) and Pseudomonas (20%). PROM >48 hrs had a 100% positive blood culture compared to 33.33% in PROM >24 hrs and 16.86% in PROM>18 hrs. Foul smelling liquor has 100% positive septic screen and a 37.5% positive blood culture. Sepsis screen test is bedside test, simple, cheap and less time consuming.

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