Original Research Article

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A study of bacteriological profile and outcome of babies born to mother with Premature rupture of membrane and its correlation with blood and gastric culture

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ABSTRACT

Background: PROM, a condition that occurs when fetal membranes are ruptured at least one hour before onset of labor. While PROM is observed in 10% of all pregnancies, 60-80% of PROM is observed in term and 20-40% in pregnancies less than 37th gestational week. PROM is the most significant reason for preterm labor. The three causes of neonatal death associated with PROM are prematurity, sepsis and pulmonary hypoplasia. Infants born with sepsis have a mortality rate four times higher than those without sepsis. Objective of the study was correlation of blood and gastric culture positive sepsis in PROM newborns. It helps to find out the incidence of PROM in our locality.

Methods: This retrospective study enrolled 90 neonates born to healthy mothers with history of PROM more than 18 hours duration, admitted in SNCU/ward at a tertiary care hospital for six months duration from 1st January 2017 to 30 June 2017. Clinical profile of these Newborn with history of PROM was noted such as birth weight, gender, gestation, duration of membrane rupture, history of maternal fever. For all newborns with PROM, sepsis screen had been sent. The neonatal outcome was also recorded, and the data was collected and analyzed by using frequency and percentages.

Results: Gram negative bacilli were the commonest cause of neonatal sepsis and male neonates were more prone to infection. PROM and low birth weight especially, ELBW and VLBW are the common high-risk factors for early onset sepsis. Most common organisms isolated in blood and gastric culture were *Klebsiella* and *Staphylococcus aureus* respectively.

Conclusions: PROM is a high-risk obstetric condition. Active management is needed to enable delivery within 24 hours of PROM as it offers better neonatal outcome. Morbidity and mortality increase as the duration of PROM increases. This can be reduced by early diagnosis, specific treatment and strict infection control practices in neonatal units.

Keywords: Blood culture, Gastric culture, Neonates, Micro-organisms, Outcome, Premature rupture of membrane

INTRODUCTION

Premature rupture of membrane (PROM) is a condition that occurs when fetal membranes are ruptured at least one hour before the onset of labor and amniotic fluid flows out so, the barrier between fetus and external environment is broken down. If it occurs before 37th

gestational week, it is called preterm premature rupture of membrane (PPROM). PROM and PPROM are similar in terms of their etiologies, complications and outcomes; however, it is considered that the actual cause of membrane rupture in PPROM is the infection around choriodecidual region. While premature rupture of membrane is observed in 10% of all pregnancies, about

60-80% of PROM is observed in term pregnancies and 20-40% of PROM is observed in pregnancies lower than 37th gestational week. PPROM is observed in 2-3% of all pregnancies and it is the most significant reason for preterm labor. Spontaneous preterm labors before 32nd gestational week are frequently accompanied by clinical or subclinical infection symptoms. It is together with long-term morbidity in newborns. The incidence rate is quite high in subsequent pregnancies.

The diagnosis of PPROM is made by clinical suspicion, patient history and simple testing. Patient history has an accuracy of 90% for the diagnosis of PPROM and should not be ignored.³ Numerous tests have been recommended for the evaluation of PPROM but two tests have withstood the test of time: nitrazine (Bristol Myers Squibb, Princeton, New Jersey) paper testing and ferning (also referred to asamniotic fluid crystallisation testing) of the vaginal pool. Freidman and McElin found that if a combination of patient history, nitrazine testing and ferning were used to evaluate a patient for PROM, the accuracy of at least two positive tests was 93.1%. During diagnosis and evaluation of PPROM, a digital cervical examination should be avoided. Lewis et al, compared the digital cervical examination with the sterile speculum examination and found that latency was shortened significantly by cervical examination at any gestational age (2.1 [4.0] vs 11.3 [13.4] days, p<0.0001).2

The three causes of neonatal death associated with PPROM are prematurity, sepsis and pulmonary hypoplasia. Infants born with sepsis have a mortality rate four times higher than those without sepsis.⁴ Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first month of life. Early neonatal sepsis implies that the infection presented at or before first 72 hours of life. Neonates with sepsis may have nonspecific signs and symptoms including temperature instability, hypotension, poor perfusion with pallor, mottled skin and respiratory distress.² The risk for developing neonatal sepsis increases progressively with the time elapsed between rupture of membranes and eventual delivery. A five-fold rise in sepsis is seen when comparing incidences at 24 hours versus 72 hours of premature rupture of membranes. Neonatal screening can be carried out using clinical guidelines as well as selected laboratory investigations. Serum C-reactive protein levels have been shown to be highly sensitive and specific for neonatal sepsis.

The present study aims at correlation of blood culture positive sepsis in PROM newborns and also the correlation of gastric culture with PROM. It is helps to find out the incidence of PROM in our locality.

METHODS

This retrospective study enrolled total 90 neonates born to healthy mothers with history of PROM and PPROM of more than 18 hours duration, admitted in SNCU and/or ward at a tertiary care hospital during the period of six

months from 1st January 2017 to 30th June 2017. This study was approved by the institutional ethics committee. Mothers with PROM of more than 18 hours duration who have received antibiotics prior to delivery, antepartum hemorrhage, toxemia of pregnancy, co-morbidities in the mother other than infection, neonates with major congenital anomalies were excluded from the study.

Clinical profile of these Newborn with history of PROM was noted such as birth weight, gender, term/preterm, duration of rupture of membrane, history of maternal fever. All the newborns with PROM, sepsis screen had been sent for analysis for CBC, CRP and blood culture. At the same time gastric wash was given and was sent for analysis for gastric culture. Newborn was started on empirical antibiotics. Other symptoms such as respiratory distress, neonatal jaundice, hypoglycemia etc. treated, if present, as per standard protocol. The case Performa is filled after parents' consent. The blood culture and gastric culture were recorded. The neonatal outcome was also recorded in terms of survival of child (death/discharged). The data was collected and analyzed by using frequency and percentages.

RESULTS

Total 90 neonates with PROM were enrolled in the study, among them 62(68.88%) were male child and 28(31.11%) were female child. Distribution of male and female neonates in different birth weights is shown in Table 1.

Total number of term neonate was 48(53%) and preterm neonates were 42(47%), (Table 2). Blood culture growth was positive in 54(60%) neonates among them 35(83%) were preterm and 19(38%) were term neonate with PROM while gastric culture was positive in 16(18%) cases among them 4(10%) were preterm and 12(25%) were term neonates with PROM. Contaminant growth was found in one (2%) term neonate (Table 2).

Table 3 show the microbial growth in blood culture and gastric culture in preterm and term newborn with PROM. The most predominant micro-organism grown in blood culture was *klebsiella* in 15(17%) cases, followed by E. coli and *staphylococcus aureus* in 10(11%) neonates each while in gastric culture *Staphylococcus aureus* was commonly grown in 7(8%) cases followed by coagulase negative *staphylococcal aureus* in 4(5%) cases.

The blood culture was positive in all of the two (100%) of the extremely low birth weight (ELBW), it was positive in 19(95%) out of 20 very low birth weight (VLBW). Also, it was positive in 23(58%) out of 40 of low birth weight (LBW) and 9(32%) neonates with normal weight (that of between 2.5 kg to 4 kg) had positive blood culture and was contaminant in one case of same weight group. The correlation of blood culture growth in different birth weight is shown in Table 4. History of duration of rupture of membrane was studied

with respect to blood culture positivity are shown in Table 5.

Table 1: Distribution of neonates as male and female child in different birth weights.

Neonates	Different birt	Different birth weights				
reonates	ELBW	VLBW	LBW	Normal Total		
Male Child	1 (1.61%)	13 (20.96%)	27 (43.54%)	21 (33.87%)	62 (68.88%)	
Female Child	1 (3.57%)	07 (25%)	13 (46.42%)	7 (25%)	28 (31.11%)	
Total	2 (2.22%)	20 (22.22%)	40 (44.44%)	28 (31.11%)	90 (100%)	

Table 2: No of term and preterm new-born with PROM having growth in blood culture and gastric culture.

Neonates	Blood culture		Contaminant	Contaminant Gastric Culture		— Total
Neonates	Positive	Negative	Contaminant	Positive	Negative	Total
Preterm	35 (83.33%)	7 (16.66%)	0 (0.00%)	4 (9.52%)	38 (90.47%)	42(100%)
Term	19 (39.58%)	29 (60.41%)	1 (2.08%)	12 (25.0%)	36 (75.0%)	48 (100%)
Total	54	36	1	16	74	90

Table 3: Microbial growth in blood culture and gastric culture in preterm and term new-born with PROM.

Microbial growth in blood	l culture	No. of patients	Microbial growth in gas	tric culture	No. of patients
Stanbulasasaya ayraya	Preterm	05 (9.25%)	Staphylococcus aureus	Preterm	02 (12.5%)
Staphylococcus aureus	Term	05 (9.25%)		Term	05 (31.25%)
E. coli	Preterm	05 (9.25%)	Coagulase negative	Preterm	01 (6.25%)
E. Cott	Term	05 (9.25%)	staphylococcal aureus	Term	03 (18.75%)
Kleibsiella	Preterm	12 (22.22%)	E. coli	Preterm	-
Kielosiellä	Term	03 (5.55%)		Term	02 (12.5%)
Pseudomonas	Preterm	05 (9.25%)	Kleibsiella	Preterm	-
Pseudomonas	Term	01 (1.85%)		Term	01 (6.25%)
Coagulase negative	Preterm	03 (5.55%)	Pseudomonas	Preterm	01 (6.25%)
staphylococcus aureus	Term	01 (1.85%)		Term	-
Staphylococcal	Preterm	4 (7.40%)	Candida	Preterm	-
epidermidis	Term	=		Term	01 (6.25%)
Citrobacter	Preterm	01 (1.85%)	-	Preterm	-
Curobacier	Term	01(1.85%)		Term	-
Enterococci	Preterm	-	-	Preterm	-
Enterococci	Term	01 (1.85%)		Term	-
Stranta a a a a u a vivi di ana	Preterm	-	-	Preterm	-
Streptococcus viridians	Term	01 (1.85%)		Term	-
Contaminant	Preterm	-	-	Preterm	-
Contaminant	Term	01 (1.85%)		Term	-
Total		54		Preterm	16

Table 4: Microbial growth in blood culture in different birth weights of new-born with PROM.

Microbial growth in blood culture	ELBW	VLBW	LBW	Normal
Staphylococcus aureus	-	4(20%)	4 (10%)	2 (7.14%)
E. coli	1 (50%)	3 (15%)	4 (10%)	2 (7.14%)
Kleibsiella	-	5 (25%)	9 (22.5%)	1 (3.57%)
Pseudomonas	1(50%)	2 (10%)	2 (5%)	1 (3.57%)
Coagulase negative staphylococcus aureus	-	1 (5%)	2 (5%)	1(3.57%)
Staphylococcal epidermidis	-	3 (15%)	1 (2.5%)	-
Citrobacter	-	1 (5%)	1 (2.5%)	-
Enterococci	-	-	-	1 (3.57%)
Streptococcus viridians	-	-	-	1 (3.57%)
Contaminant	-	-	-	1 (3.57%)
Total (out of)	2 (100%)	20 (100%)	40 (100%)	28(100%)

Table 5: Correlation of duration of rupture of membrane and blood culture and gastric culture positive sepsis in new-born with PROM.

Blood culture	<18 hour	18-24 hour	25-48 hour	>48 hour
Positive	14 (53.84%)	24 (57.14%)	9 (64.28%)	7 (87.5%)
Negative	12 (46.15%)	18 (42.85%)	5 (35.71%)	1 (12.5%)
Total	26 (100%)	42 (100%)	14 (100%)	8
Gastric culture	<18 hour	18-24 hour	25-48 hour	>48 hour
Positive	7 (26.92%)	7 (16.66%)	1 (7.14%)	1 (12.5%)
Negative	19 (73.07%)	35 (83.33%)	13 (92.85%)	7 (87.5%)
Total	26 (100%)	42 (100%)	14 (100%)	8 (100%)

Table 6: Correlation of duration of rupture of membrane and microbial growth in blood and gastric culture in new-born with PROM.

Microbial growth in blood culture	<18 hour	18-24 Hour	25-48 hour	>48 Hour
Staphylococcus aureus	2 (7.69%)	6 (14.28%)	-	2 (25%)
E. coli	1 (3.84%)	6 (14.28%0	2 (14.28%)	1 (12.5%)
Kleibsiella	4 (15.38%)	7 (16.66%)	4 (28.57%)	-
Pseudomonas	2 (7.69%)	1 (2.38%)	-	3 (37.5%)
Coagulase negative staphylococcus aureus	2 (7.69%)	2 (4.76%)	-	-
Staphylococcal epidermidis	2 (7.69%)	-	2 (14.28%)	-
Citrobacter	-	1 (2.38%)	-	1 (12.5%)
Enterococci	-	1 (2.38%)	-	-
Streptococcus viridians	-	-	1 (7.17%)	-
Contaminant	1 (3.84%)	-	-	-
No growth	12 (46.15%)	18 (42.85%)	5 (35.71%)	1 (12.5%)
Microbial growth in gastric culture	<18 hour	18-24 Hour	25-48 hour	>48 Hour
Staphylococcus aureus	4 (15.38%)	2 (4.76%)	-	1 (12.5%)
Coagulase negative staphylococcal aureus	-	4 (9.52%)	-	-
E. coli	1 (3.84%)	1 (2.38%)	-	-
Kleibsiella	1 (3.84%)	-	-	-
Pseudomonas	1 (3.84%)	-	-	-
Candida	-	-	1 (7.17%)	-
No growth	19(73.07%)	35 (83.33%)	13 (92.85%)	7 (87.5%)

Table 6 shows the correlation of duration of rupture of membrane and microbial growth in blood and gastric culture in newborn with PROM Out of 90 newborn 15 (16.6%) were died and 75 were discharged / DAMA. Deaths among preterm newborn with PROM were 8/42 (19%) that of in term neonate with PROM were 7/48 (14.5%). Only two neonates with PROM in current study were ELBW and which were died, thus, the 100% mortality in ELBW. Deaths among VLBW were 5/20 (20%). Deaths in LBW were 4/40 (10%); and that of in normal weight was 4(14%) out of 28 normal weight babies. Deaths among newborn with PROM having positive blood culture were 11(20.4%) while positive gastric culture were 1 (6.25%), (Table 7). Deaths among positive microbial blood culture were studied and it was found that mortality was 50% in pseudomonas growth (3 out of 6 died). It was 40% in case of E.coli growth (4 out of 10 died); 25% in case of coagulase negative staphylococcus aureus(1 out of 4 died) and 20% in case of staphylococcus aureus (2 out of 10 died).

In case of kleibsiella growth in blood culture death was 6.66 % (1 out of 15 died). No death occurred in spite of blood culture showing microbial growth in blood culture in case of streptococcus viridians, staphylococcal epidermidis, enterococci, citrobacter and contaminant growth, (Table 8). Deaths among positive microbial gastric culture were studied and it was found that mortality was 50% in E. coli growth (1 out of 2 died). No death occurred in spite of gastric culture showing microbial growth in gastric culture in case of staphylococcus aureus, coagulase negative staphylococcus aureus, kleibsiella, pseudomonas, candida. Deaths were 14 (19%) in negative gastric culture, (Table 8). Deaths were studied with respect to duration of rupture of membranes and it was found that deaths were 4 (15.38%), 6(14.28%), 1(7.14%), 4(50%) in duration of rupture of membrane of <18 hours, 18-24 hours, 25-48 hours and greater than 48 hours respectively, (Table 9).

Table 7: Correlation of no. of neonate with PROM with positive blood and gastric culture and their outcome.

Blood culture	Deaths	Discharge/DAMA	Total
Positive	11 (73.33%)	43 (57.33%)	54
Negative	4 (26.66%)	32 (42.66%)	36
Total	15 (100%)	75 (100%)	90
Gastric culture	Deaths	Discharge/DAMA	Total
Positive	1 (6.66%)	15 (20%)	16
Negative	14 (93.33%)	60 (80%)	74
Total	15 (100%)	75 (100%)	90

Table 8: Correlation of outcome of new-born with PROM W.R.T. blood culture and gastric culture growth.

Microbial growth in blood culture	Deaths	Discharge/DAMA	Total
Staphylococcus aureus	2 (13.33%)	8 (10.66%)	10
E. coli	4 (26.66%)	6 (8.0%)	10
Kleibshella	1 (6.66%)	14 (18.66%)	15
Pseudomonas	3 (20%)	3 (4.0%)	6
Coagulase negative staphylococcus aureus	1 (6.66%)	3 (4.0%)	4
Staphylococcal epidermidis	-	4 (5.33%)	4
Citrobacter	-	2 (2.66%)	2
Enterococci	-	1 (1.33%)	1
Streptococcus viridians	-	1 (1.33%)	1
Contaminant	-	1 (1.33%)	1
No growth	4 (26.66%)	32 (42.66%)	36
Microbial growth in gastric culture	Deaths	Discharge/DAMA	Total
Staphylococcus aureus	-	7 (9.33%)	7
Coagulase negative staphylococcal aureus	-	4 (5.33%)	4
E. coli	1 (6.66%)	1 (1.33%)	2
Kleibshella	-	1 (1.33%)	1
Pseudomonas	-	1 (1.33%)	1
Candida	-	1 (1.33%)	1
No growth	14 (93.33%)	60 (80%)	74

Table 9: Outcome of neonates with PROM in various duration of rupture of membrane.

Duration of rupture of membranes	Deaths	Discharge /DAMA	Total
<18 hour	4 (15.38%)	22 (84.61%)	26 (100%)
18-24 hour	06 (14.28%)	36 (85.71%)	42(100%)
25-48 hour	1 (7.14%)	13 (92.85%)	14(100%)
>48 hour	4 (50%)	04 (50%)	8(100%)

DISCUSSION

In the present study it was found that the incidence of sepsis was higher in males than females. Several other workers have reported similar finding.⁵⁻⁸ Bias for male sex, place of study, sample including other factors may be responsible for increased number of male cases in these studies. Though the exact reason for this male preponderance is not known with certainty, it is probably due to the fact that the factors regulating the synthesis of Y-globulins are situated on the X-chromosome.^{9,10} Since

the male has only 1 X-chromosome, he is less immunologically protected than the females. The incidence of sepsis was higher in low birth weight neonates when compared to normal birth weight babies, this finding was correlated with the previous studies. ^{6,11-13} This increase incidence is because of low birth weight babies have low IgG levels as well as impaired cellular immunity and hence are more susceptible to infection. A systematic analysis of global, national and regional causes of child mortality in 2013 identified preterm birth complications and infections to be the two major causes

of neonatal deaths in India.¹⁴ Kifah and Al-Awayshah study found that incidence of PROM was more in preterm gestation while according to Doyle, 70% of cases of PROM occurred at term and 30% of PROM was preterm.^{15,16} The present study also found increased incidence of PROM in term gestation (53%), this results are consistent with Lokhande et al, study.⁸

Total 54 neonates were blood culture positive, giving a positivity rate of 60%, where most predominant microorganism grown was *klebsiella*, followed by *E. coli* and *staphylococcus aureus*. This result was comparable with the other studies. ^{14,17-19} The gastric culture positivity rate was 18%, where *staphylococcus aureus* was commonly grown micro-organism. Contaminant growth occurred in one neonate. The culture positivity depends on time of sampling, extent of bacteremia in neonate and prior antibiotic treatment in the neonate. Gram negative organisms formed the majority of the isolates as compared to Gram positive organisms in the present study, comparable with the study done by Lazarus et al. ¹⁸

The correlation of blood culture growth in different birth weight show that blood culture positivity rate was more in low birth weight (LBW) when compared to neonates with normal weight (that of between 2.5kg to 4kg). In recent years, there has been a lot of improvement in medical facilities and as a result, the survival rate of the preterm and LBW babies has improved. But at the same time, these neonates with immature immune defenses are exposed to NICU flora for a longer duration. Most of the neonatal septicemia cases now are either LBW or preterm.²⁰

History of duration of rupture of membrane was studied with respect to blood culture positivity. Duration of rupture of membrane was divided into 4 groups as less than 18 hours, 18-24 hours, 25-48 hours, greater than 48 hours. Blood culture was positive as 14(54%), 24(57%), 9(62%) and 7(88%) whereas gastric culture was positive as 7(30%), 7(17%), 1(7%), 1(12.5%) cases in duration of rupture of membrane of <18 hours, 18-24 hours, 25-48 hours and greater than 48 hours respectively. There was increased in microbial growth observed in blood culture when the duration of rupture of membrane increases. Highest microbial growth observed in >48 hours of duration (87.5%) and lowest growth observed in <18 hours of duration (53.84%) of PROM. In contrast to blood culture, in gastric culture there was decreased in microbial growth observed when increasing the duration of rupture of membrane. Highest growth observed in <18 hours of duration (26.66%) and lowest growth observed in 25-48 hours (7.14%) of duration of PROM.

Concerning outcome, 15(16.6%) babies were diedwhich was comparable with the study done by Chiabi et al, in which they reported 22% mortality.²¹ Among 15 deaths 8 were preterm and 7 were term neonate with PROM. According to the WHO, low birth weight neonates have 20 times more risk of dying than those of normal weight.²² In present study, also mortality was higher in

low birth weight babies than the normal weight. Deaths among newborn with PROM having positive blood culture were 11(20.4%) and that of in negative blood culture were 4(11.11%). *Pseudomonas, Escherichia coli* and *Staphylococcus aureus* were the most frequent germs responsible for the deaths; these findings are similar to the studies done by Chiabi et al, and Cisse et al.^{21,23}

These studies and current study demonstrate that the germs usually responsible for mortality are those which are most resistant to empirical treatment. The relation between duration of rupture of membranes and death were studied and it found that more death (50%) were observed in the >48 hour of PROM. This finding revealed that mortality in neonates born to mothers with PROM is directly related to the duration of PROM. Nili and Ansari observed that mortality in one group with PROM <24 hours is less than mortality in one group with PROM >24 hours.²⁴

CONCLUSION

Premature rupture of membranes is a high-risk obstetric condition. Active management is needed to enable delivery within 24 hours of premature rupture of membranes as it offers better neonatal outcome. Gram negative bacilli were found to be commonest cause of neonatal sepsis and male neonates were more prone to infection. PROM and low birth weight especially, extremely low birth weight (ELBW), and very low birth weight are the common high-risk factors for early onset sepsis.

Most common organisms isolated in blood culture were *Klebsiella* while in gastric culture were *Staphylococcus aureus*. Morbidity and mortality increase as the duration of premature rupture of membranes increases. This can be reduced by early diagnosis, specific treatment and strict infection control practices in neonatal units.

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