MICROBIOLOGICAL STUDY ON ENDOCERVIX IN PRETERM PREMATURE RUPTURE OF MEMBRANE

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ABSTRACT

BACKGROUND

Preterm premature rupture of membrane (PPROM) is defined as premature rupture of membrane before 37 completed weeks. It is associated with 40% preterm deliveries and results in significant perinatal mortality and morbidity. Present study is an attempt to find the association between infection and PPROM.

MATERIALS AND METHODS

100 pregnant women between 29 weeks and 34 weeks of gestation who were admitted in our labour room during a period from November 2012 to November 2013 were included. Preterm Premature Rupture of Membrane (PPROM) is confirmed by history, sterile per speculum examination demonstrating pooling of fluid in posterior vaginal fornix and vaginal pH. An ultrasound examination showing oligohydramnios also supports the diagnosis.

RESULTS

62% of neonates had RDS; p value <0.001, strong significance. 16% had no morbidity. 10% had late sepsis. 6% had NHB; p value 0.090, moderate significance. 6% had PHTN.

CONCLUSION

Relation between infection and PPROM remains an association. So patients at risk for preterm delivery need to be watched more closely for infection as it is also associated with neonatal morbidity.

KEYWORDS

PPROM, Oligohydramnios, Malpresentations, Amnionitis.

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BACKGROUND

Preterm Premature Rupture of Membrane (PPROM) is defined as premature rupture of membrane before 37 completed weeks. It is associated with 40% preterm deliveries and results in significant perinatal mortality and morbidity. Infants born with sepsis have mortality rate four times higher than those without sepsis. Present study is an attempt to find the association between infection and PPROM and the neonatal outcome.

Aims and Objectives

To study the spectrum of microorganisms in endocervical canal in preterm premature rupture of membrane and study the perinatal outcome.

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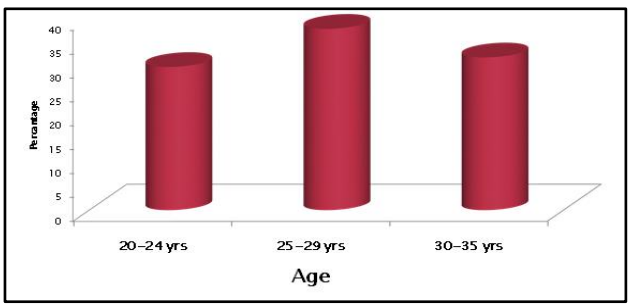
MATERIALS AND METHODS

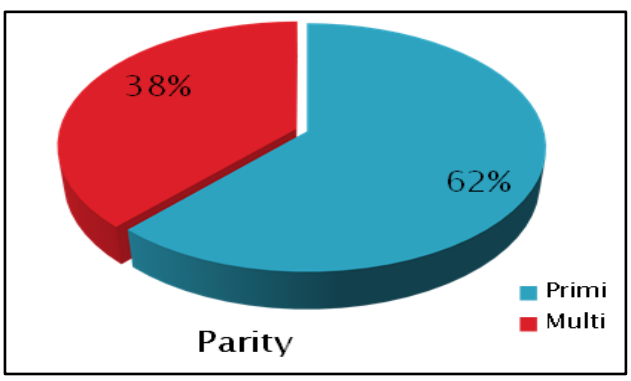
- 100 pregnant women between 29 weeks and 34 weeks of gestation who were admitted in our labour room during a period from November 2012 to November 2013.
- Preterm premature rupture of membrane (PPROM) is confirmed by history, sterile per speculum examination demonstrating pooling of fluid in posterior vaginal fornix and vaginal pH. An ultrasound examination showing oligohydramnios also supports the diagnosis.
- Endocervical swab is taken from cervical canal under sterile conditions by sterile technique.
- The swabs taken were inoculated in blood agar, chocolate agar and MacConkey's for 24 – 48 hours. Culture is considered sterile if no growth is seen after 72 hours.
- Laboratory tests were conducted to identify group B Streptococci, Candida, Klebsiella, Pseudomonas, E. coli.
- Newborns were followed up in NICU.

RESULTS

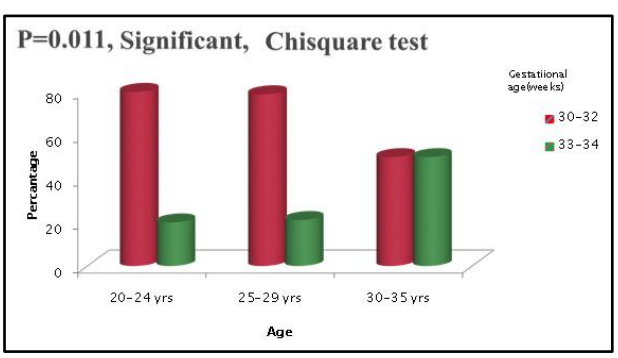
- 62% of neonates had RDS; p value <0.001, strong significance.
- 16% had no morbidity.
- 10% had late sepsis.
- 6% had NHB; p value 0.090, moderate significance.
- 6% had PHTN.



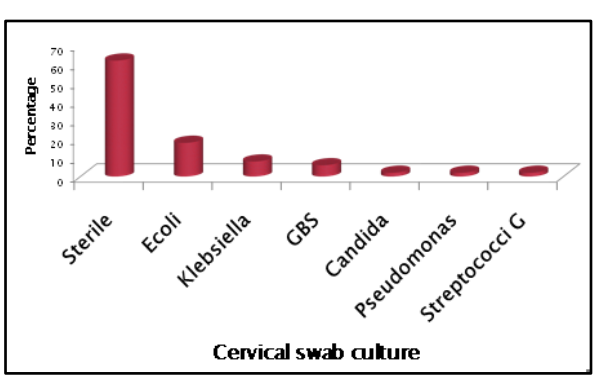
Graph 1. PPROM was more Commonly seen in Age Group 25-29 Years Accounting to 38%



Graph 2. Primigravidae had more Incidence of PPROM (62%) than Multigravidae

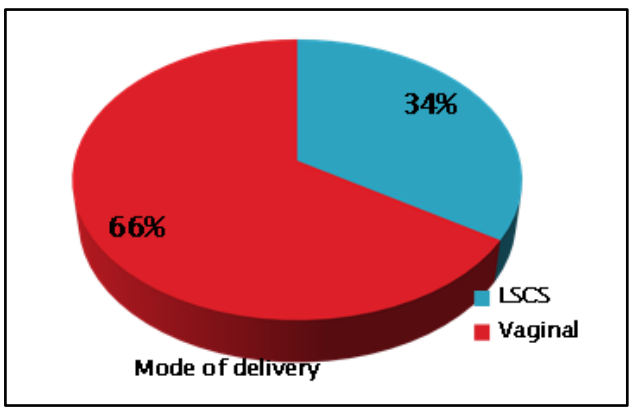


Graph 3. PPROM was more Common in Gestational age of 30-32 Weeks

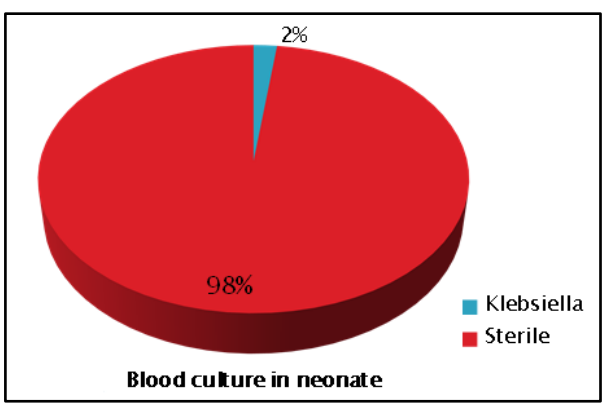


Graph 4. Cervical Swab Culture of Patients Studied

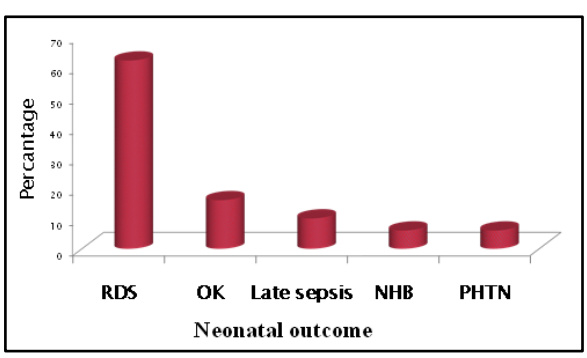
E. coli was positive in 18%, Klebsiella in 8%, GBS in 6%, Candida in 2%, Pseudomonas in 2% and Streptococci G in 2%. Significant correlation of infection with preterm premature rupture of membranes, mainly Klebsiella (P value 0.010).



Graph 5. Mode of Delivery of Patients Studied



Graph 6. Blood Culture in Neonates



Graph 7. Neonatal Outcome of Patients Studied

Statistical Methods

1. Descriptive and inferential statistical analysis has been carried out in the present study.
2. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%).
3. Chi-square/Fisher Exact test has been used to find the significance of study parameters.

DISCUSSION

A total of 100 patients with preterm premature rupture of membranes with gestational age between 29 weeks to 34 weeks of gestation were included in the study.

Malpresentations, multifoetal gestation, polyhydramnios, PPROM due to prenatal diagnostic techniques like amniocentesis, cordocentesis, iatrogenic causes like cervical encirclage were excluded.

We found a significant correlation with infection and preterm premature rupture of membranes, mainly Klebsiella (P value = 0.010) and Candida (P value= 0.088). 62% of cases were primi. 70% were between 32-34 weeks. 34% of cases went for LSCS and 66% had vaginal delivery. 98% of neonates had a sterile blood culture and 2% got Klebsiella positive. We found that 62% of the neonates had RDS (P value ≤0.001). Late sepsis in 10% (p value =0.643), Neonatal hyperbilirubinaemia in 6% (p value=0.090) and pulmonary artery hypertension in 6%.

Limitations of study were, antibiotics were given to mothers presented with PPROM after taking the endocervical swabs. So neonatal outcomes were better even in mothers with a positive culture report.

Rupture of membrane is the most distinctive clinical feature of membrane activation. One of the biochemical marker of membrane activation of is an increase in collagenolysins. Collagen may be degraded as a result of several matrix metalloproteinases (MMP). The activity of MMP-9, MMP-1, MMP-8 are elevated in women with bacterial invasion of amniotic membrane.[1] Intra-amniotic infections may cause alterations in tensile strength of foetal membrane by mechanism different from those involved in the increase in collagenolytic activity. Several of the microorganism found in women with PPROM produce proteolytic enzymes that can weaken the foetal membranes.[2] The changes in antibacterial properties of cervical mucus also play a role in facilitating ascending infections.[3] Onderdonk, Delaney et al[4] did a microbiologic study as a part of larger project which collected 1365 placental samples obtained after delivery. The tissue was cultured and PCR methods with bacterial primers were used. Mycoplasma, Ureaplasma, Enterobacter species were present in the culture. Reisenberger K observed that some organisms induce cytokine production from amniocytes in a concentration dependent manner.[5] Sbarra AJ and others postulated that the acidic environment from infection destabilises endogenous lysosomal membranes empowering bacteria to be more effective in deactivation of immunoglobulin A and G in cervical mucus.[6] Group B Streptococci was first recognised as the cause of neonatal septicaemia by Dunham in 1933.[7]

The pathogenesis of PPROM is not completely understood. Several factors are thought to lead to rupture of membrane and one of which is the presence of infectious agent as postulated by Regan.[1] Collagen may be degraded as a result of several matrix metalloproteinase (MMP) that bind to specific tissue inhibitors (TIMP).The activity of MMP-9 is markedly elevated in women with bacterial invasion of amniotic membrane. Similar evidence has been obtained for MMP-1.

Intra-amniotic infections may cause alterations in tensile strength of foetal membrane by mechanisms different than those involved in the increase in collagenolytic activity. Several of the microorganisms found in women with PPROM and amniotic fluid infection produce proteolytic enzymes that can weaken the foetal membranes.[4] The host inflammatory response includes the production of cytokines that stimulate prostaglandin production by amnion and chorion. The evidence implicating infection as an aetiology agent in membrane weakening and membrane rupture is robust. The microorganisms isolated from amniotic fluid cultures (Peptostreptococcus, Bacteroides, Fusobacterium, Ureaplasma urealyticum, etc.,) are similar to those normally found in vagina, strongly suggesting that the source of infection is vaginal flora. The mechanism of ascending infection is unclear. Under normal circumstances, the membranes are separated from vaginal flora by cervix and cervical mucus. The changes in antibacterial properties of cervical mucus also play a role in facilitating ascending infection.

In vitro studies have been performed that investigated the effect of bacterial collagenases, elastases and proteases on amniotic membrane. Certain bacteria are capable of producing enzymes that directly degrade collagen and its matrix. Gregar JA and Mcgregar JA found that Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus agalactiae, Bacteroides and Enterobacteriaceae all produce collagenases.[2] In a study done by Reisenberger K, he observed that some organisms induce cytokine production from amniocytes in a concentration dependent manner.[5]

Sbarra AJ and others postulated the resulting acidic environment destabilises endogenous lysosomal membranes, empowering bacteria to be more effective in deactivation of immunoglobulin A and G in cervical mucus. Ultimately it results in reduced mucous volume by increased mucinase activity, now allowed by altered immunologic environment. The amnion itself can respond to bacteria by producing cytokines. The important cytokines, IL-6 and IL-8 are most likely produced by polymorphonuclear leukocytes. Until recently maternal origin of origin of leukocytes response was only presumed, however, foetal origin of these cells has been documented now. In a study done by Sampson JE, he found that the foetal leukocytes also produce IL-1b, another cytokine, in concentration exceeding that of adult cells.[8] Foetal IL-1b induces production of PGE2 which is a potent oxytocic which increases uterine contractility and increases chance of membrane rupture. It is presumed that a messenger signal to the foetus, most likely is produced in response to bacteria in a pathologic state or as a part of maturational process in normal pregnancy, stimulates the foetal production of Type 5 collagenases with the disruption of basement membrane, both the water tight seal and bacteriostatic barrier are compromised. This was postulated by Newton ER and et al.[9] Colonisation of lower genital tract by Chlamydia, Neisseria, Trichomonas and Bacteroides species increases the risk of rupture of membranes before term. Acute chorioamnionitis is a severe complication of PPROM. The most commonly found organism in women with acute chorioamnionitis are GBS and E. coli which are present in 20% of cases and responsible for 67% of maternal or foetal bacteraemia. GBS was first recognised as the cause of neonatal septicaemia by

Dunhan in 1993.[7] In 1980, Miller JM et al studied bacterial colonisation of the amniotic fluid with intact foetal membranes.[10] Amniotic fluid was collected from 45 selected patients by amniocentesis or needle anatomy prior to or during labour or by needle aspiration at the time of caesarean section. Fluid cultured and examined directly by Gram stain. Among 14 who were not in labour, only one had growth of bacteria. Among amniotic fluid cultures from 31 patients in labour, 13 were positive (>10^2 CFU/mL) on primary plating media, five grew in broth only and 13 were negative. Of the 13 patients with positive culture, six contained only aerobes, five contained only anaerobes, and two contained mixed aerotolerance type. Aerobic bacteria included Haemophilus influenza, Pseudomonas aeruginosa, Klebsiella pneumonia, Listeria monocytogenes, Group B beta haemolytic Streptococci. Presence of bacteria on Gram stain and >10^2 CFU/mL from culture were significantly associated with the presence of clinical chorioamnionitis. In 1989, a study was done by Edward R, Newton et al to identify risk factors for intra-amniotic infection. In a pilot study, stepwise logistic regression identified duration of ruptured membrane as significant risk factor for intra amniotic infection. Using the preliminary regression equation, they established risk criteria to predict probability of intra-amniotic infection. Subsequently, 2908 patients were screened for risk criteria and or the presence of intra-amniotic infection. 24% met the risk criteria. In patients meeting risk criteria, the relative risk of intra-amniotic infection was 19.7. In addition, 12% of mothers developed endometritis. Ten neonates of mothers with criteria developed sepsis or pneumonia. Intense inflammatory reaction at the site of prematurely inflamed membranes is noted as early as 1950. Several studies have shown an association between Group B Streptococci. Some studies have not been able to find any correlation. Sweet et al in a study of 3293 women were not able to demonstrate an association between Group B Streptococci and PPROM.[11]

In a study by Achari et al and Toth, the common organism isolated were Staphylococcus albus (19%).[12] The less common being Staphylococcus aureus, E. coli, GBS and anaerobic Streptococci. They also found an increased incidence of perinatal mortality and morbidity with increasing duration of ruptured membranes (>48 hrs.). They were more in patients of lower socioeconomic group. Russel postulated that sepsis is first manifested as amnionitis, the premature rupture of membranes accompanied by rise of temperature to 38^0 Celsius or more before or during labour and escape of malodorous amniotic fluid with no other focus of infection. Lanier observed that when labour does not ensue within 24 hours 0.50% developed amnionitis and 28.5% got postpartum infection.[13]

Larry C Gilstrap et al studied in 1988 on 312 patients with acute chorioamnionitis and impact on neonatal sepsis. 90 women received antibiotics after cord clamping and 70 did not receive antibiotics. Antibiotics were administered during labour rather than after cord clamping if delivery was not imminent. Although endometritis developed more frequently in patients who received antibiotics after cord clamping, it was not statistically significant. (5.6% vs. 3.9%). There were 2 cases of verified infection in the group of infants born to mothers who received intrapartum antibiotics and 8 cases in non-antibiotic group (p=0.005). They concluded that administration of antibiotics to mother after rupture of membranes decreased incidence of neonatal sepsis. Gram stain of amniotic fluid is a reliable test for detection of intra-amniotic infections with a specificity of 98.6.[6] In women with PPROM, a positive Gram stain has 93.3% PPV and negative Gram stain has 85.4% NPV for diagnosis of intrauterine infection. If Gram stain is negative for bacteria and WBC, the probability of infection is less than 5%. The National Institute of Child Health and Human Development Maternal-Fetal Medicine Units research network performed a large prospective observational study to identify the risk factors for preterm premature rupture of membrane. One of the risk factors was bacterial vaginosis (RR: 1.5;95% CI, 1.0-2.1). D Guilo DB, Romero R, Kusanovic et al studied the prevalence and diversity of microbes in amniotic fluid in PPROM. In this study, 34% of amniotic fluid samples had polymicrobial invasion like Bacteroides, Fusobacterium, Enterococcus, Peptostreptococcus, Staphylococcus, Streptococcus, Candida, etc.

Mercer BM, Goldenberg RL et al studied about the prediction of preterm premature rupture of membrane through clinical findings and ancillary testing under The National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network, found significant association between bacterial vaginosis and PPROM.

A study conducted in extreme preterm premature rupture of membranes (<26 weeks) by Al Riyami N and his colleagues published in Oman Medical Journal shows overall concurrent infection rate was high among those patients. Study on epidemiological microbiological aspects of cervicovaginal infections in pregnancy conducted by Benedetto C, Tibaldi C et al showed significant association between cervicovaginal infections and PPROM (p<0.0001). Cervicovaginal cultures were positive in 84.6% of PPROM. Kacerovsky M, Pliskova l et al conducted a study on the correlation of microbial load of mycoplasma to the degree of histologic chorioamnionitis in PPROM. 103 cases were studied. The presence of genital mycoplasmas in amniotic fluid was found in 38% of women.

A study on clinical and microbiological correlation of PPROM conducted by C Karat, P Madhivanan, K Krupp et al is published in Indian Journal of Medical Microbiology. Out of 150 cases studied, 39% of cases had WBC in vaginal fluid showing presence of infection. There was significant association between infection and PPROM. Bacterial vaginosis (OR:10.5,95% CI:3.5,31.5), E. coli (OR:7.5:95% CI:2.8,20.0), Staph aureus (OR:2.8:95% CI:1.24,3.6), 4 cases of Group B Streptococci and 4 cases of P. mirabilis were positive. In Thai Journal of Obstetrics and Gynaecology, there is an Epidemiological study of cervical swab culture in PPROM conducted at Ramathibodi Hospital by Angkharn Trinity MD, Somsak, Pratakv to investigate incidence and results of cervical swab culture in PPROM. The most common organism was Gardnerella vaginalis (34.8%), Group B Streptococci (6.8%). A study published in the

Journal of Evolution of Medical and Dental Sciences on cervical swab cultures to predict premature rupture of membrane shows significant association of infection and premature rupture of membrane. Prevalence of cervicovaginal infection found were 27% in first trimester, 28% in second trimester and 29% in third trimester. Common organisms found were Streptococci, Candida and Staphylococci. And treating these infections antenatal was helpful in reducing the preterm births. Banerjee S, Sanyal S, Banerjee U et al published a study on histology and bacteriological profile in premature rupture of membrane. Out of 102 cases studied, culture done in vaginal swab, cervical swab and amniotic fluid showed predominant presence of E coli, Streptococcus haemolyticus, Klebsiella, Staph aureus. A study conducted in the University Hospital in Prague published in Acta Medica provides evidence of association between genital mycoplasma and PPROM. Calleri lF, Taccani C, Porcelli A published a study on the correlation between PPROM and genital infections. 302 vaginal swabs were taken and domination of Ureaplasma Urealyticum was found. An analysis of microorganisms in cervical canal of subjects who gave birth prematurely in Poland done by Midwifery Division Faculty in Medical University of Warsaw showed Enterococci faecalis infection occurred significantly in pregnancies complicated by PPROM. In a study on Group B Streptococci and PPROM published in American Journal of Obstetrics and Gynecology, 60 patients with PPROM were included in the study and 15 had Group B Streptococci culture positive. Kacerovsky M, Musiloval, Khatibi A et al studied intra-amniotic inflammatory response to bacteria in PPROM. They did polymerase chain reaction in amniotic fluid and bacteria was found in 435 of women.

Limitations of Study

Antibiotics were given to mothers who presented with PPROM after taking the endocervical swabs. So neonatal outcomes were better even in mothers with a positive culture report.

CONCLUSION

Relation between infection and PPROM remains an association. So patients at risk for preterm delivery need to be watched more closely for infection as it is also associated with neonatal morbidity.

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