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Original Research Article

Study of vaginal flora in women with premature rupture of membrane and it's association with maternal and perinatal outcome.

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Abstract:

Background: Normal vaginal flora that is lactobacillus maintains the vaginal PH thus preventing women from day to day infection. Premature membrane rupture puts the mother and fetus at risk for complications such maternal infection, fetal infection, umbilical cord compression, cord prolapse, fetal death, poor Apgar score, pulmonary hypoplasia, preterm delivery, low birth weights, and fetal deformities. Our aim is to study spectrum of vaginal flora in women with premature rupture of membrane and it's association with maternal and perinatal outcome.

Methodology: A prospective observational study was conducted in 128 women admitted with premature rupture of membrane in obstetric wards of R D Gardi Medical College, Ujjain; over one year. All pregnant women with gestational age more than 28weeks admitted in obstetrics ward having complaint of leaking per vagina were included in this study. Swab were collected for culture and sensitivity to laboratory.

Result: Mean age was 26.28 ±4.12 years, and median age was 27 years. In high vaginal swab cultures, growth was observed in 73 women (57.0%). E.coli was the commonest observed organism (22.7%) followed by Staphylococcus aureus (14.1%). Mixed growth was seen in 8.6% women . Maternal outcome was fever (10.9%), UTI (9.4%), puerperal sepsis(3.1%), chorioamnionitis(4.7%) and wound infection(4.7%). Neonatal outcome were NICU admissions , Prematurity, Low Birth Weight and Neonatal Sepsis in 35.6%, 19.2% ,28.8% ,6.8% respectively.

Conclusion: In this study we conclude that 57% women had abnormal vaginal flora in pregnant women presenting with PROM and were linked with adverse pregnancy outcome and adverse neonatal outcome. We conclude that microbiologic screening in early pregnancy may aid in the assessment of patient risk for preterm delivery.

Keyword: Vaginal flora, PROM, Maternal outcome, Perinatal Outcome

1. INTRODUCTION:

Among women of reproductive age, genital infections are linked to serious morbidities, particularly during pregnancy[1]. Normal vaginal flora such as lactobacillus maintains the vaginal PH thus prevents women from day to day infection. Disturbance in the vaginal flora with the overgrowth of bacteria that are present in small numbers such as Gardnerella, Prevotella, Mobiluncus, Peptostreptococcus etc and decrease in lactobacillus causes high PH and clue cells generally known as Bacterial vaginosis [2]. Bacterial vaginosis during

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pregnancy increases the risk of histopathological chorioamnionitis, early miscarriage, preterm premature rupture of membranes, spontaneous preterm labour, and post sepsis. Endotoxin secreted by microorganisms along with the residual reaction may cause premature membrane rupture by inducing uterine contractions and weakening the fetal membrane[3]. Premature membrane rupture puts the mother and fetus at risk for complications such as maternal infection, fetal infection, umbilical cord compression and prolapse, fetal death, poor Apgar score, pulmonary hypoplasia, preterm delivery, low birth weights, and fetal deformities [4]. Worldwide the prevalence of PROM ranges between 2 and 10 % [5]. In India Prevalence of PROM ranges between 10-40% [6] The main maternal risk is chorioamnionitis, which affects 35% of pregnancies. Other complications are abruption in 19% of pregnancies, and sepsis in 1% of pregnancies [7]. The normal development and outcome of pregnancy depends on the fetal membranes' structural integrity and functionality. Relevance of this study is to find scenario of bacteria which are responsible for chronic chorioamnionitis resulting in PROM.

2. METHODOLOGY:

This is a prospective observational study to assess maternal and neonatal outcome in PROM women on the basis of presence of growth in vaginal swab culture. The study duration was one year, conducted in women admitted with premature rupture of membrane in obstetric wards of C.R. Gardi Hospital, Ujjain.128 women of more than 28 week gestation who had complaint of leaking per vaginum and diagnosis of premature rupture of membrane confirmed by clinical examination were included in this study. A detailed history was taken from all study subjects along with detailed clinical examination and was recorded in their case proforma. Patient's medical history, family history, history of blood transfusion, diabetes mellitus, cigarette smoking, genital infections, poor nutrition and past obstetric history of rupture of membrane was also recorded. Taking proper antiseptic measures, Sim's speculum was inserted to retract the posterior vaginal wall and anterior vaginal wall retractor was used to retract anterior wall of vagina to look for any visible leaking. If no leaking is present, patient is asked to cough or slight pressure on the uterus is given to confirm the absence or presence of leakage. With the help of vaginal swab stick we collect the high vaginal sample and send it for culture to microbiology department. Vaginal fluids are cultured on three primary culture media: blood, chocolate and McConkey agar. Cultures are incubated at 37^0 C for 24 hours. Gram staining was done for diagnosis. Reports were obtained in 24-48 hour.

Data analysis: The data was recorded in Microsoft excel sheet and then transferred to SPSS software for analysis. Cross tables and charts were made and chi square was calculated. p value was calculated wherever possible. The protocols were approved by Institutional Ethics Committee, No 45/2021.

3. RESULT :

Demographic characteristics

In this study, a total of 128 participants were enrolled. Mean age was 26.28 ±4.12 years, and median age was 27 years. Out of 128 women, 64.8% were belonging from rural area. Most women were illiterate and belongs to lower socio economic status as seen in table 1.

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Table 1: Demographic features of study participants

Age Groups	**Frequency (%)**
<= 20 Years	16 (12.5%)
21 - 30 years	95 (74.2%)
> 30 Years	17 (13.3%)
Locality	
Rural	83 (64.8%)
Urban	45 (35.2%)
Education	
Illiterate	69 (53.9%)
Undergraduate	55 (42.9%)
Graduate	4 (3.2%)
Occupation	
Daily wages	73 (57%)
Housewife	55 (43%)
Socio economic status	
Lower	58 (45.3%)
Upper lower	44 (34.4%)
Upper middle	26 (20.3%)
Total	128 (100%)

Gestational age

It was observed that 79 females (61.7%) presented with less than 37 weeks of gestation and 49 females (38.3%) presented with more than 37 weeks of gestation.

Table 2: Gestational age and PROM

Gestational age	**Frequency(%)**
Preterm	79 (61.70%)
Term	49 (38.3%)

Duration of rupture of membranes

In the study it was observed that 56.3% had less than twelve hour and 43.8% had more than twelve hour duration of rupture of membranes

Table 3: Duration of rupture of membranes

Duration of rupture of membranes	**Frequency(%)**
<12 hour	72(56.2%)
>12 hour	56(43.8%)

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Type of organism

In high vaginal swab cultures, E.coli was the commonest observed organism (22.7%) followed by Staphylococcus aureus (14.1%). Mixed growth was seen in 8.6% women as seen in table 4

Table 4: High vaginal swab culture and type of organism

Types of organism	Frequency (%)
Candida	6 (4.7%)
Citrobacter	5 (3.8%)
E.coli	29 (22.7%)
Klebsiella sp.	3 (2.3%)
Mixed growth	11 (8.6%)
Staphylococcus aureus	18 (14.1%)
Streptococcus sp.	1 (0.8%)
No growth	55 (43%)

Maternal outcome :

In those who had growth, 19.20% women had fever, 16.4% had UTI, 12.3% had Chorioamnionitis, 2.70% had puerperal sepsis, 5.50% had Wound infection. PPH was seen in 1.40% women. In present study significant association was found between maternal complications fever, UTI, PPH, chorioamnionitis and high vaginal swab culture with $p < 0.05$.

Table 5: Correlation of maternal outcome with growth in culture

Maternal complication		**High vaginal swab culture**		**Chi-square**	**p**
		Growth	No Growth		
		N %	N %		
Fever	Yes	14 (19.20%)	0 (0.00%)	11.843	0.001
	No	59 (80.80%)	55 (100.00%)		
UTI	Yes	12 (16.40%)	(0.00%)	9.976	0.002
	No	61 (83.60%)	55 (100.00%)		
Chorioamnionitis	Yes	9 (12.30%)	1 (1.80%)	4.81	0.026
	No	64 (87.70%)	54 (98.20%)		

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Puerperal sepsis	Yes	2 (2.70%)	2 3.60%	0.083	0.773
	No	71 (97.30%)	53 (96.40%)		
Wound infection	Yes	4 (5.50%)	2 (3.60%)	0.238	0.625
	No	69 (94.50%)	53 (96.40%)		
PPH	Yes	1 (1.40%)	5(9.10%)	4.185	0.041
	No	72 (98.60%)	50 (90.90%)		

Neonatal outcome

In those who had growth, NICU admission was seen in 35.6% , Prematurity in 31.50% , Low Birth Weight in 28.8% and Neonatal Sepsis in 6.8% cases.

Table 6: Correlation between neonatal complications and growth in culture

Neonatal complications		**High vaginal swab culture**		**Chi-square**	**P**
		Growth N %	No Growth N %		
NICU Admission	YES	26 (35.60%)	24 (43.60%)	0.848	0.357
	NO	47 (4.40%)	31 (56.40%)		
Prematurity	YES	23 (31.50%)	17 (30.90%)	0.005	0.942
	NO	50 (68.50%)	38 (69.10%)		
Low birth weight	YES	21 (28.80%)	17 (30.90%)	0.069	0.793
	NO	52 (71.20%)	38 (69.10%)		
Neonatal sepsis	YES	5 (6.80%)	1 (1.80%)	1.777	0.183
	NO	68 (93.20%)	54 (98.20%)		
Neonatal jaundice	YES	14 (19.20%)	8 (14.50%)	0.473	0.492
	NO	59 (80.80%)	47 (85.50%)		
Pulmonary hypoplasia	YES	3 (4.10%)	1 (1.80%)	0.544	0.461
	NO	70 (95.90%)	54 (98.20%)	0.267	0.605
Birth asphyxia	YES	6 (8.20%)	6 (10.90%)		
	NO	67 (91.80%)	49 (89.10%)		

4. DISCUSSION:

The harmony and interactions of vaginal microbes are essential to female vaginal health. The vaginal lactobacilli seen in the majority of healthy women play a crucial part in protecting the host from genital tract infections[8]. One of the significant risk factors for PROM is vaginal infections that are bacterial, fungal, or mixed infection during the third trimester of

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pregnancy[9]. Vaginitis can be diagnosed swiftly by the Nugent score particularly in BV[10]. Pathogens like group B streptococci are readily detected by culture method [11] or through microscope such as vaginal candidiasis[12]. However, early-stage bacterial vaginitis cannot be diagnosed by the Nugent score. And also, the culture method may not be able to cover all species of pathogen[13].

128 study subjects were investigated by high vaginal swab culture to find the presence of pathogenic organism and their effect on maternal health. In present study we found that mean age of pregnant women came with complaint of leaking PV was 26.2 year. PPROM was seen common in age group below 25 years as per other studies done by Noor et al. 19 showed 58.8%.However, in our study we found that maximum cases are seen in 26-30 year age group (45.3%) followed by 21-25 year age group(28.9%). It was observed that many of them belonged to rural area. And many of them belong to lower socioeconomic class. Pandey S et al suggesting that lower socioeconomic factor has a influential role in the existence of preterm labour.[14]

Incidence

In present study incidence of altered vaginal flora is 57%. Similar incidence were being observed in other studies [15].

Table 7: Abnormal vaginal flora in PROM reported by various authors

S.N.	Author , year	Incidence of AVF
1.	Do Youn Kwon et al. 2021	21.4%
2.	Rani S et al, 2014	62%
3.	Baqui AH et al, 2018	16.5%
4.	Gondo F et al , 2011	45.7%
5	Sule-Odu AO et al , 2020	33.3%
6	Tibaldi C et al , 2016	43.8%
7	Present study	57%

Micro-organism:

E-coli was the commonest organism seen in the study done by other authors [16-19]. Similarly, in present study E Coli (22.7%) was the commonest organism isolated in culture followed by Staphylococcus aureus (14.1%). Low-virulence organisms that become vulnerable early in pregnancy cause these infections connected to PTB, which can result in chronic intrauterine infections even in the absence of overt clinical infection symptoms. [20]. However, once inside the uterine cavity, they cause the synthesis of pro-inflammatory cytokines, prostaglandins, and metalloproteases. These can result in premature preterm birth (pPROM), cervical ripening and shortening, weakening of the membranes, and rupture [21].

Maternal complication:

Maternal complication in women presenting with complaint of per vaginum leaking, fever (19.20%) was commonly reported, followed by UTI (16.40%) while chorioamnionitis (12.3%) , PPH(1.40%),puerperal sepsis (2.70%) and wound infection (4.7%) respectively were reported in present study. This was in opposing with the studies done by other authors [22 -23] where endometritis was the most common complication seen. In another study they found out that the incidence of puerperal sepsis was 8.68%[24].

Table 8: Comparison of prevalence of various maternal complications

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Complications	Lanier Jr etal (2015)	Kaur T et al (2016)	Tachawatcharapunya S et al (2017)	Mahajan C et al (2020)	Present study (2020-2021)
Chorioamnionitis	20%			3.57%	12.3%
Puerperal sepsis		8.68%			1.4%
PPH				13.39%	1.4%
PROM			17.8%		39.1%

It was observed that in present study maternal complication like chorioamnionitis had found growth in high vaginal swab (12.3%). A study conducted by lanier Jr et al, the incidence of chorioamnionitis after PROM is 20%.[25] In different studies, the incidence of chorioamnionitis was reported from 7.5% to 37.5% [26, 27].

Neonatal complication:
In present study we found that new born of mother with positive vaginal swab culture, 35.6% of new born were admitted in NICU, 31.5% were born preterm, 28.80% were born with low birth weight, 6.8% were diagnosed as neonatal sepsis,19.20% newborn diagnosed with neonatal jaundice, 4.10% newborn born with pulmonary hypoplasia and 8.20% of newborn had birth asphyxia. Although they were not significantly associated but many studies discussed that compared to women with a healthy vaginal microbiota, pregnant women with BV had approximately a four times increased risk to deliver a neonate with LBW and a six times increased risk to deliver a preterm neonate with LBW. Similar independent relationships between BV and PTB-LBW were discovered by Hillier and colleagues in a multicenter research from the United States (aOR, 1.4). Similarly, BV was found to be strongly and independently linked with both LBW (aOR, 2.0) and PTB-LBW in a large Danish cohort research (aOR 2.5). [28]

5. CONCLUSION :

This study reports a high incidence of abnormal vaginal flora in pregnant women presenting with PROM and were linked with adverse pregnancy outcome and adverse neonatal outcome. incidence of altered vaginal flora in our study setting is 57%. E Coli (22.7%) was the predominant organism isolated in culture followed by Staphylococcus aureus (14.1%). Maternal complications like fever, UTI, PPH and chorioamnionitis were found to be significantly ($P<0.05$) associated with positive swab culture from the high vaginal swab specimen. This shows a positive correlation between pathogenic organisms and maternal adverse effects.

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