Clinical Evaluation And Analysis Of Cervicovaginal Exudate Samples In Pregnant Women With Preterm Premature Membrane Rupture

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Citation

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Abstract

Objective: To evaluate genital microbial isolation pattern by analyzing cervicovaginal exudates in women with preterm premature rupture of membranes (PPROM) diagnosis and its relationship with the clinical aspects and gestational age of pregnancy.

Materials and methods: Prospective, observational study in a high specialty hospital. We included patients with pregnancy between 20- and 36-weeks' gestation (WG) with diagnosis of PPROM, obtaining demographic and clinical data, in addition to performing cervicovaginal exudate for microbial identification.

Results: 62 patients were included, 25 (40%) presented positive cervicovaginal exudate samples, with Candida albicans being the most commonly identified pathogen. Median age 28.5 (95% CI 27-30) years, with pregnancy of 33.8 (95% CI 32.1- 34.5) WG, gestations of 2 (95% CI 2-3) and 16 (95% CI 10-26) hours of rupture of membranes' evolution. The absence of abnormal vaginal discharge was associated with negative vaginal exudate culture (p = 0.02). Previable pregnancy had a higher proportion of germ isolation significantly (p = 0.01). There was an association of positive cervicovaginal exudate with gestational age for pregnancies under 24 WG (p = 0.008).

Conclusion: Those patients who present the PPROM with pregnancy less than 24 WG have a higher rate of positive cervicovaginal exudate samples.

INTRODUCTION

Premature rupture of membranes (PROM) refers to the rupture of fetal membranes before the start of labor. When this occurs before 37 weeks' gestation (WG) we call it preterm premature rupture of membranes (PPROM) [1]. This condition occurs between 2 and 3% of all pregnancies. Approximately 0.5% of pregnancies are less than 27 WG, 1% of 27 to 34 WG and another 1% of 34 to 37 WG; in addition, PPROM is responsible for almost a third of preterm births [2,3]. We can classify the PPROM into three groups: near term (32 to 36 weeks), remote term (24 to 31 weeks) and previable (less than 24 weeks) [4].

The most common maternal complications due to PPROM include: chorioamnionitis, endometritis, compression or prolapse of the umbilical cord and premature detachment of the placenta [5,6].

Premature birth is the main cause of death in children under 5 years, and about 30% are caused or preceded by PPROM [7]. Neonatal complications are mainly due to prematurity and they include respiratory distress syndrome, intraventricular hemorrhage, necrotizing enterocolitis, retinopathy of prematurity, bronchopulmonary dysplasia, low birth weight, restriction deformities and sepsis [8,9].

To this day, the etiology of PPROM is not clear, but multiple risk factors that can trigger it have been described, among which we can mention: genitourinary infections, antepartum transvaginal bleeding, polyhydramnios, acute trauma, short

cervix, obstetric antecedent of PROM, poor nutrition and smoking [10,11,12,13].

The inflammatory process, produced by genital infections, could cause weakening of the fetal membranes in the pregnant woman triggering a premature rupture of the membranes [14,15].

Genital infections by Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Gardnerella vaginalis, Group-B Streptococcus (GBS) and colonization by Escherichia coli have been associated with PPROM [16-21]. Its association with candidiasis is controversial; one study showed a reduction in PPROM by administering treatment for this yeast [22].

The purpose of the study was to determine the pattern of genital microbial isolation by analyzing cervicovaginal exudates in women diagnosed with PPROM and its relationship with clinical aspects and gestational age of pregnancy in a high specialty hospital.

MATERIALS AND METHODS

Study design and patient selection.

An observational, prospective study was conducted in the High Specialty Medical Unit # 48, Hospital of Gynecology and Pediatrics, of the Mexican Social Security Institute, in the city of León, Guanajuato, Mexico. This unit is a third-level care hospital, with 345 censable beds, of which 122 are exclusively intended for obstetric and gynecological care, taking care of more than 12,000 births a year, being a reference center for cases with PPROM.

The data was collected since October 1, 2018 to April 30, 2019, with the previous authorization of the Health Ethics Committee and Local Health Research Committee, with registration number R-2018-1002-37, in accordance to the guidelines of the Declaration of Helsinki. All patients signed an authorization through informed consent.

Women over the age of 18 were included, who came to the hospital for medical attention with a diagnosis of PPROM and pregnancy between 20 and 36 weeks with six days of gestation. The diagnosis of PROM was made from the history of vaginal fluid drainage that moistened genitals and ran to the thighs and legs, complemented with the examination with sterile speculum, by observing accumulation of fluid in the posterior vaginal fornix or free flow of fluid from the cervix. In addition, all patients underwent confirmatory crystallography, which consists of performing a smear of the liquid on a glass foil and letting it dry for 10 minutes and so it can be viewed later under a microscope, being considered positive when an image is observed in ferns. We do not include pregnant women with caloric-protein malnutrition, neither those who had received antibiotics in the last seven days, nor patients with urinary tract infection or other identifiable infectious focus, nephropathies, rheumatic diseases, chronic degenerative pathologies such as diabetes mellitus or chronic arterial hypertension, HIV / AIDS infection or viral hepatitis (A, B, C), transvaginal hemorrhage, diagnosis of polyhydramnios, cervical isthmic incompetence or positive smoking during pregnancy.

Procedures.

Demographic and clinical data of each patient were taken, such as age, weight, height, number of pregnancies, deliveries, caesarean sections or previous abortions, precedent of PPROM in previous pregnancies, and the last menstruation date in order to estimate the weeks of gestation according to the latter, which was corroborated in all cases by ultrasound. Time and date of the rupture of membranes and its medical diagnosis were recorded, as well as symptoms of genital infection (abnormal vaginal discharge, pruritus, burning or vaginal fetidness) in case they were present in the patient.

Upon admission and confirmation of PPROM, cervicovaginal exudate samples were taken; a sterile vaginal speculum was placed until the cervix was observed. Subsequently, the sampling was carried out as follows: a vaginal cavity and cervix scan was performed with two sterile cotton swabs. Immediately, the first swab was rubbed against a urine test strip to determine the pH; afterwards, the same swab, is rubbed against a glass foil (slides) to perform Gram staining and was observed under the microscope in search of gram positive or gram-negative bacteria in addition to Candida yeasts. Finally, the swab is introduced in a sterile tube with 1.5 ml of 0.9% saline solution, then it is placed on a slide and observed under the microscope (wet mount test) for the diagnosis of *Trichomonas vaginalis,* in addition to determining the presence or absence of *Clue* cells or *Candida* yeasts. The second swab was used to immerse it in a tube with *Stuart* microbiological transport medium for laboratory shipment and culture obtainment. The cervicovaginal exudate samples were processed in the unit's microbiology laboratory and were transported immediately.

With the second swab, the sample was seeded in the following culture media: Blood Agar, BiGGY Agar, MacConkey Agar, Thayer-Martin Agar and Salted Mannitol Agar. The samples were incubated for a period of 72 hours at a temperature of 34-37oC.

For the diagnosis of *Gardnerella vaginalis*, the Amsel criteria were used: 1) Presence of Clue cells, 2) Vaginal pH over 4.5, 3) White-gray vaginal discharge, 4) Positive Whiff test or KOH test, of which three are necessary for diagnosis.

72 hours later, after the sowing of the sample, the result was obtained, which described the microorganism identified in case of being positive.

Due to logistical problems and availability of culture medium for its isolation, *Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum* and *Herpes simplex* virus were not reported.

Statistical analysis

The results are expressed in median and 95% CI. The differences between clinical data and gestational age groups, according to the vaginal exudate report, were evaluated using 2 and Fisher's exact test for proportions. Mann-Whitney U test was used since the variables did not show normal distribution. The cumulative influence of the positive report of vaginal exudate, with gestational age and clinical variables was investigated by logistic regression analysis considering entering a variable if $p < 0.05$ and eliminating it if $p > 0.1$. Significance was considered with a value of p less than 0.05. The statistical analysis was performed with the NCSS statistical software (Copyright © 2019 NCSS) and Epidat version 4.2 (www.sergas.es/Saude-publica/EPIDAT).

RESULTS

A total of 62 pregnant patients with PPROM were included in the study. No patient was excluded. The values of the variables are expressed in median and 95% confidence intervals (95% CI), since they did not present normal distribution. The patients' age was 28.5 (95% CI 27-30) years, with a pregnancy of 33.8 (95% CI 32.1-34.5) weeks of gestation; weight of 72 (68-75) kg and height of 1.58 (95% CI 1.56-1.60) m, with BMI of 29.1 (95% CI 27.3-30.3) kg/m2. As for the gestations, the median was 2 (IC9% 2-3), with only 8 (12.9%) being primigravidae patients and the remaining multigravidae.

At the time of confirmation of the rupture of membranes, the median was 16 (95% CI 10-26) hours of evolution; 27 (43.5%) cases had> 24 hours of PPROM evolution.

From the total of analyzed patients, only in 25 (40%) cases, some germ was isolated. A total of eight different types of germs were identified, with Candida albicans being the most common, as shown in Table I.

The analyzed clinical variables of patients with positive and negative cervicovaginal exudate were compared, finding only a significant difference in the number of deliveries, being higher in the group with negative culture (Table II).

When analyzing the clinical characteristics, only the absence of abnormal vaginal discharge was significantly associated with a negative culture of vaginal exudate (Table III).

When comparing the patients, according to the gestational age, we observed that all the patients with a previable pregnancy had positive cultures, unlike those of remote term and near term pregnancy, significantly (Table IV).

With bivariate analysis, an association of positive cervicovaginal exudate with gestational age was demonstrated for pregnancies under 24 WG (Table V).

There was no significant difference when comparing the cultures of patients of <24 hours of PPROM evolution with those of > 24 hours (p=0.64). The distribution for the first group was as follows: positive cervicovaginal culture, n=15, 24.1% and negative culture, n=20, 32.2%; and for the second group, positive culture, n=10, 16.1% and with negative culture, n=17, 27.4%.

A multivariate analysis was performed with logistic regression without demonstrating a significant association of positive cervicovaginal exudate with other variables such as gestational age, body mass index, patient age, abnormal vaginal discharge, pruritus, burning and vaginal fetidness, hours of membrane rupture evolution and history of PPROM.

DISCUSSION

PPROM entails multiple maternal and fetal complications [5-9] and the genital infections have been associated as a probable cause of it [16-22].

In the present study, 40% of the vaginal exudates of the analyzed patients were positive. A very similar result to the one reported in a study carried out in a Nigerian hospital, in which they reported 44% of cervicovaginal exudates with

isolation of some pathological germ in pregnant women with PPROM [19], similar to that described in another study conducted in Uganda, in which 52% of vaginal cultures were positive [23].

Various microbial agents were detected in the group of patients studied, such as Candida albicans, Escherichia coli, Gardnerella vaginalis, Enterococcus spp, Corynebacterium vaginale, Candida glabatra, Staphylococcus aureus and Klebsiella pneumoniae, in order of frequency, with the most frequently isolated germ being Candida spp, in more than half of the cases with positive exudate (Candida albicans in 52% of cases and Candida glabatra in 4%). However, it is important to mention that, a control group without PPROM was not included, since it would be necessary to obtain cultures from asymptomatic pregnant women, paired for gestational age, which would imply ethical issues. When comparing our results with other investigations, it was observed that the frequency of isolation is well above that recorded in other studies such as Aboyeji A. P. et al. [19], with 23%; Nakubulwa S. et al. [24], with 20%; Eleje G. U. et al. [25], with 8.5% and Zhang L. X. et al. [26] with 6% of cases. This may be due to the fact that in Mexico the most common genital infections are due to Candida and Gardnerella vaginalis, just as reported in another study carried out in our country on non-pregnant women with recurrent genital tract infections, in which both germs were main causes of such infections [27].

In the study by Rasti S. et al. [28], candidiasis was not associated with RPMP, although other authors have concluded that having candidiasis has been a protective factor for PPROM [24, 25, 26]; in the case of the patients analyzed in the present study, 22% of cases with PPROM presented it.

We detected that Escherichia coli was the second pathogen isolated in frequency, similar to the studies carried out by Celen S et al. [29] and Saghafi N. et al [30], conducted in Turkey and Iran, respectively, which report this bacterium as the main isolated pathogen in cases with PPROM, associating colonization of this bacterium with PPROM in both studies. According to the systematic review done by Zeng Ln et al., carried out in China, in which they identified the main microbial agents associated with the PROM in that country, Escherichia coli was the second most isolated germ. In this same study Corynebacterium and Enterococcus were the main gram-positive bacteria isolated, unlike our study where those were presented in low proportion [31].

In his study, Aboyeji A. P. et al., managed to isolate Gardnerella vaginalis as the main pathogen and associated it with PROM [19]; instead, in our study it had a very low frequency of appearance.

Finally, Staphylococcus aureus had a very low isolation frequency, with only 4% of cases, unlike other studies in which they report it as the most isolated bacterium in vaginal cultures in patients with PROM [23, 31].

It is important to mention that no Streptococcus was identified in our study, despite having the means available to isolate it and the conditions in the patients. In some studies, it is reported as the most frequently isolated germ and is associated with PPROM [25, 26]. In contrast, there are other studies that, like us, did not report isolation of this germ [19, 24].

Although some studies have reported an association of infection with Trichomonas vaginalis [18,24] and Neisseria gonorrhoeae [17] with PPROM, in the present study it was not possible to isolate these pathogens, probably because they are uncommon in genital infections in Mexico [27].

In relation to the gestational age of presentation of the PPROM, we can show that our data coincide with the one described in the literature, since the lowest frequency is found in previable pregnancies and two thirds corresponded to pregnancies near term [3]. If we refer to parity in our study group, we found that the median number of gestations was 2, in the group of positive cultures, coinciding with that described by Eleje GU et al., where his group with PPROM had an average parity of 0 to 2 pregnancies [25].Adding this to our study, patients with a history of higher numbers of vaginal deliveries tend to have fewer positive cervicovaginal exudates.

There are few studies which include previable pregnancies and analyze vaginal exudates, such as that of Rasti S. et al. [28]. In our study we include 5 cases of pregnancies with less than 24 weeks, of which in its totality a germ was isolated. It is important to mention that the majority of the studies focus on remote or near term gestational ages.

It would be interesting in future research to carry out a study which includes a greater number of patients in equitable groups by gestational age (predictable, remote and near term) with greater control of the variables, in order to be able to analyze more accurately the association of specific isolated germs with the rupture of membranes.

In relation to abnormal vaginal discharge, the absence of it is significantly associated with a negative culture, the above mentioned differs from that reported in the studies of Al-Hussain T. K. et al. [32] and Assefa N. E. et al. [33] where they manage to associate abnormal discharge with infection and PPROM.

The vaginal symptomatology evaluated in our study as pruritus, burning or fetidness was not associated, as a whole, with positive cervicovaginal exudates. However, 19% of the patients studied presented vaginal fetidness, a percentage very similar to that reported of 15% in the study of Al-Hussain TK et al., in their group of patients with PPROM, where they manage to associate this clinical condition with PPROM [32].

It has been reported that the history of PPROM in previous pregnancies is associated with a new event of PPROM in the current pregnancy [12]; in our case this history was present only in 22.5% of patients, similar to that reported in studies of Al-Hussain T.K. et al. [32] and Assefa N. E. et al. [33].

We were unable to associate the hours of evolution of the PPROM with positive cervicovaginal exudates, unlike from what was reported by the study of Musaba M. W. et al [23].

It is important to mention that in this study patients without potential triggers of PPROM, or that could influence the result of the analysis of cervicovaginal exudate were included, as mentioned in the inclusion criteria.

Another point to consider in future projects would be to establish the appropriate conditions regarding ethical aspects, to include a control group of patients without PPROM, with paired gestational age. Also, have resources for the isolation of germs such as Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum and Herpes simplex virus. It is convenient to consider analyzing a greater number of patients with gestational ages of less than 24 weeks gestation, since it is where a greater proportion of positive cervicovaginal exudates is identified.

Table 1

Germs isolated in pregnant women with PPROM.

Case number	WG at the time of PPROM diagnosis	Hours of evolution of PPROM	Isolated germ
1	35.6	0.5	*Candida albicans*
3	36.1	20	*Enterococcus spp*
8	32.6	15	*Candida albicans*
9	32	12	*Candida albicans*
12	30	32	*Corynebacterium spp*
13	36.5	1.5	*Staphylococcus aureus*
14	23.4	0.5	*Candida albicans*
15	35.6	15	*Candida albicans*
16	36.1	12.5	*Corynebacterium vaginale*
17	34.1	22.5	*Escherichia coli*
21	36.4	7.5	*Candida albicans*
26	23.3	26	*Candida albicans*
27	21.2	2	*Gardnerella vaginalis*
30	34.1	72	*Klebsiella pneumoniae*
31	36.4	28	*Escherichia coli*
41	36.4	6	*Candida albicans*
42	34.4	53	*Escherichia coli*
43	23.1	10	*Candida albicans*
45	21.3	52	*Candida albicans*
46	35.1	51	*Enterococcus spp*
50	35.2	1	*Candida albicans*
51	29.5	72	*Candida glabrata*
53	29.3	72	*Gardnerella vaginalis*
61	28	72	*Candida albicans*
62	36.5	11	*Candida albicans*

PPROM = Preterm premature rupture of membranes; WG= Weeks' gestation.

Table 2

Clinical characteristics comparison of patients with positive and negative cervicovaginal exudate.

Variable	Positive cervicovaginal exudate n=25	Negative cervicovaginal exudate n=37	*p*
Maternal Age (years)	28 (25-33)	29 (27-31)	0.85
Weeks' gestation (weeks)	34.1 (29.5-35.6)	33.5 (32.1-34.5)	0.80
Weight (Kg)	74 (66-78)	69 (68-77)	0.94
Height (m)	1.56 (1.54-1.60)	1.59 (1.56-1.60)	0.31
BMI (kg/m^2)	29.3 (27.3-32.8)	28.8 (27-30.3)	0.81
Gestations	2 (2-3)	3 (2-3)	0.31
Births	0 (0-0)	1 (0-1)	0.01
Abortions	0 (0-1)	0 (0-0)	0.10
Caesarean sections	0 (0-1)	0 (0-1)	0.82
PPROM hours	15 (10-32)	17(8-31)	0.95

The results are shown in medians with 95% confidence intervals; Mann-Whitney U test.

BMI= Body mass index, kg= kilograms, m= meters, kg/m^2 = kilogram over square meter, PPROM = Preterm premature rupture of membranes.

Table 3

Clinical characteristics of pregnant patients with PPROM regarding the cervicovaginal exudate sample.

Variable	Positive cervicovaginal exudate n= 25	Negative cervicovaginal exudate n=37	*P*
Abnormal vaginal discharge			
Yes	21 (33.8%)	21 (33.8%)	0.02
No	4 (6.4%)	16 (25.8%)	
Vaginal pruritus			
Yes	4 (6.4%)	6 (9.6%)	1.00
No	21 (33.8%)	31 (50%)	
Vaginal burning			
Yes	3 (4.8%)	2 (3.2%)	
No	22 (35.4%)	35 (56.4%)	0.38
Vaginal fetidness			
Yes	4 (6.4%)	8 (12.9%)	0.74
No	21 (33.8%)	29 (46.7%)	
Previous PPROM			
Yes			
No	3 (4.8%)	11 (17.7%)	0.12
	22 (35.4%)	26 (41.9%)	

Values expressed in frequency and percentages. Fisher's exact test.
PPROM = Preterm premature rupture of membranes.

Table 4

Cervicovaginal exudate by gestational age groups.

Variable	Positive cervicovaginal exudate n= 25	Negative cervicovaginal exudate n=37
Previable PPROM (< 24 WG)	5 (8%)	0
PPROM remote term (24-31.6 WG)	4 (6.4%)	12 (19.3%)
PPROM close term (32-36.6 WG)	16 (25.8%)	25 (40.3%)

Values expressed in frequency and percentages. Chi Square Test (χ^2). p =0.01
PPROM = Preterm premature rupture of membranes.
WG = Weeks' gestation.

Table 5

Comparison of cervicovaginal exudates in pregnancies with gestational age < 24 WG vs > 24 WG.

Variable	Positive cervicovaginal exudate n= 25	Negative cervicovaginal exudate n=37
Pregnancy < 24 WG	5 (8%)	0 (0%)
Pregnancy ≥ 24 WG	20 (32.2%)	37 (59.6%)

Values expressed in frequency and percentages. Fisher's exact test. p=0.0082.
WG = Weeks' gestation.

CONFLICT OF INTEREST

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