ORIGINAL ARTICLE

Risk factors associated with preterm labor, with special emphasis on preterm premature rupture of membranes and severe preterm labor

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Abstract

Background: Preterm labor (PTL) can lead to preterm birth, which can cause neonatal mortality and morbidity. Preterm premature rupture of membranes (PPROM) and severe PTL (SPTL) are serious PTL subtypes. Hereby, we aimed to investigate risk factors associated with PPROM and SPTL, among Egyptian women.

Methods: In this case-control study, 117 women were enrolled without any known medical risk for PTL. The control group (n = 45) had term labor (≥37 gestational weeks), while the case group (n = 72) had PTL (<37 gestational weeks). The PTL group was subdivided into those with PPROM (n = 18) and those with intact membranes (n = 54). Fifty-two PTL women, with accurate gestational age, were subdivided into SPTL (n = 31, ≤34 gestational weeks) and mild preterm labor (MPTL; n = 21, 35-36 gestational weeks). All groups were examined for different demographic characteristics, obstetrical history, clinical signs, and vaginal and urinary tract infections. Nominal logistic regression was applied to investigate significant variables associated with PPROM and intact membranes PTL, while ordinal logistic regression was used to estimate significant variables associated with SPTL and MPTL.

Results: The final multivariate nominal model identified abortion history, heavy vaginal bleeding history, and elevated vaginal pH as significant predictors of PPROM. The same model identified age <20 years old, abortion history, heavy growth of vaginal organisms, and any growth of Gram-negative bacilli as the significant predictors of intact membranes PTL. The final multivariate ordinal model identified age <20 years old, abortion history, vaginal pH, and heavy growth of vaginal organisms as the significant predictors of SPTL and MPTL.

Conclusion: Age <20 years old, abortion history, heavy vaginal bleeding, vaginal pH, and heavy growth of vaginal organisms were reported as risk factors for PPROM and SPTL. Most of these factors are related to infection; therefore, proper infection control is recommended during prenatal and antenatal care.

Keywords: Infection; Preterm premature rupture of membranes; Risk factors; Severe preterm labor; Vaginal

1. INTRODUCTION

Preterm labor (PTL) is the labor occurring before 37 completed gestational weeks that can lead to preterm birth (PTB).[1] PTB is a leading cause of neonatal deaths and different forms of neonatal morbidities, such as neurodevelopmental impairments, cerebral palsy, retinopathy and bronchopulmonary dysplasia.[2] PTB results from multiple etiologies and can occur either spontaneously, due to PTL or preterm premature rupture of membranes (PPROM), or iatrogenic due to fetal and maternal conditions.[2]

PPROM is the spontaneous rupture of fetal membranes before 37 gestational weeks and before the labor onset and lead to PTB. In Egypt, a prevalence of 4.1% has been estimated for PPROM, with a global prevalence between 5% and 15%.[3] PPROM impact is greatest in low- and middle-income countries, where the majority of childhood deaths associated with prematurity occur.[4]

Infection is the most common maternal complications of PPROM, which in some cases can lead to maternal death. Fetal complications include infections and fetal distress, due to umbilical cord compression, respiratory distress, necrotizing enterocolitis, and interventricular hemorrhage; in addition to long-term complications such as chronic lung diseases, developmental retardation, visual and hearing difficulties, and intellectual disabilities. In few cases, fetal deaths may occur, with greater risk at earlier gestational age.[5,6] These adverse outcomes of PPROM in PTL are highly dependent on the gestational age. They are detected with significantly higher rates when labor occurs at earlier gestational age compared with intact membrane PTL cases.[7]

Many factors were reported to be associated with the occurrence of PPROM, such as infections, placental bleeding, uterine over distension (twins), previous PTB and abortion, hypertension, black race, pre-existing diabetes, age <20 and ≥35 years

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old, and smoking during pregnancy.[1,4–8] During microbial infections, vaginal bacteria ascend from the lower genital tract into the uterus.[4] Once they enter the choriodecidua, degradation of collagen-based fetal membranes begins, through direct proteolysis or activation of matrix metalloproteinases (MMPs), leading to their rupture. MMPs are group of enzymes that are activated in term birth, PPROM or in infection.[4] Alternatively, infection-associated PPROM may occur through downregulation of genes essential for tensile strength within the chorioamnion.[9]

PTL can be classified, according to the gestational age, into early or severe PTL (SPTL) at ≤34 gestational weeks and late or mild PTL at 35-36 gestational weeks. Early or SPTL is associated with greater risks of adverse outcomes than those of late PTL.[10] Several factors were reported to be associated with SPTL as microbial infections,[11] prior preterm delivery, history of placental abruption, history of fetal demise, maternal age less than 20 years old and greater than 34 years old, and history of abortion.[10]

According to WHO, around 15 million babies are born preterm each year. The complications of PTB caused the death of one million children under five years old in 2015.[12] Death before age of five years old is mainly due to prematurity and the rates of PTB are increasing globally. As the underlying mechanisms of PTL are poorly understood, identification of PTL risk factors can be used to prevent its adverse consequences.[2]

Because many of genitourinary infections are asymptomatic, underestimation of their importance may have occurred. We have recently conducted a study, on different risk factors for PTL, where we proved the association of infections caused by *Trichomonas vaginalis*, *Mycoplasma hominis*, Gram-negative bacilli, and coryneforms with PTL in Egyptian women.[13] Furthermore, only a few studies focusing on the association of different infectious agents with PTL are available. In most of these studies, only one infectious agent in relation to PTB was investigated, such as Chlamydia,[14] bacterial vaginosis,[15] trichomoniasis,[16] urinary tract infection,[17] or human papilloma virus.[18]

Here, we determined the demographic and infectious risk factors for PPROM and SPTL, being the PTL types that have the most adverse outcomes.

2. METHODS

2.1. The study group

We have previously conducted a case-control study on Egyptian women who were in labor and admitted to the Department of Obstetrics and Gynaecology, Kasr Al Aini Hospital, Cairo, Egypt.[13] The women were enrolled during a 7-month period from December 2009 to June 2010. For the purposes of this study, labor was defined as at least three uterine contractions in 10 min or 2-3 cm cervical dilatation.[19] The gestational age was determined based on the last menstrual period combined with ultrasonographic data if present. The study population (PTL group) consisted of 72 women admitted for PTL (<37 gestational weeks): 18 (25%) cases were presenting with PPROM while 54 (75%) cases were presenting in PTL with intact membranes, named intact membranes PTL group. PPROM was diagnosed based on both patients' description of fluid leakage and amniotic fluid pooling in the posterior fornix during speculum examination.[20] Only 52 women in PTL were with accurate determination of gestational age. They were subdivided into SPTL group (n = 31), with gestational age range from 23 + 0 to 34 weeks +6 days; and mild PTL (MPTL; n = 21), with gestational age range from 35 + 0 to 36 weeks +6 days.[11] The other 20 women were excluded because they had a suggested gestational age that was a border line between MPTL and SPTL groups and failed to provide accurate estimation of their last menstrual period or early ultrasonographic data; where ultrasonographic data obtained during the third trimester are not accurate enough for precise determination of gestational age.[21] Among these excluded cases, there were three cases from the PPROM group while the remaining had intact membranes PTL. The control group (term labor group [TL]) consisted of 45 control women admitted for TL without any complications of pregnancy such as PPROM, preterm contractions, or vaginal bleeding (≥37 gestational weeks). The study has been approved by Kasr Al Aini hospital ethical committee (992009).

2.2. Methodology

We have previously analyzed different risk factors associated with PTL. However, we here are further classifying the PTL group into either PPROM and intact membranes subgroups or SPTL and MPTL subgroups based on the presence of membrane rupture and on the gestational age, respectively. We used the data from our previous study in analyzing different risk factors for PTL subgroups.[13] The assignment of each participant to either TL or PTL as well her PTL type and gestational age is given in Supplementary File 1, http://links.lww.com/JCMA/A45.

2.3. Statistical analysis

The tests of two or more proportions were done using Fisher's exact test. The p values were from two-sample–tailed tests (http://www.physics.csbsju.edu/stats/exact.html). Post-hoc pairwise Fisher's exact tests were done using the rcompanion package of the R programming language to check for significant difference between any two groups.[22] Analysis of variance test was used to compare continuous data, such as age and weight between different groups, by the Minitab version 18 (Minitab Inc., State College, PA, USA).[23] This program was also used for the residual analysis to check the normal distribution of the residuals and the absence of outliers.

To summarize the outcome and exposure (variables) relationship, in our case-control study, the only measure of association that can be estimated is the odds ratios (ORs), by using logistic regression, where other measures of risks (relative or absolute risk) cannot be estimated because of the sampling method.[24] We initially conducted univariate logistic analysis to determine significant predictors of PPROM, intact membranes PTL, SPTL, and MPTL, using real statistics resource pack in the Excel program.[25] All significant predictors at a p value less than 0.050 were then included in the multivariate logistic regression model.[26] Backward elimination was then used to eliminate non-significant predictors one by one until all the predictors have a p value of less than 0.050, then we would not eliminate any predictors and the current model would be our best fitting model.[26]

Nominal logistic regression was applied to determine the significant predictors of PPROM and intact membranes PTL (outcomes have no natural ordering), while ordinal logistic regression was applied to determine the significant predictors of SPTL and MPTL (outcomes have an order). Nominal and ordinal logistic regression were performed using the Minitab 18.[23] All cases with missing values were not included in our analysis. The number of missing cases, in each model, is given in Supplementary file 2, http://links.lww.com/JCMA/A45.

3. RESULTS

3.1. The detectability of different factors in PPROM, intact membranes PTL and TL groups

3.1.1. Demographic characteristics, pregnancy history, medical complications, and speculum examination

There was no significant difference among the three groups in the mean age, weight, or height (data not shown), but women less than 20 years old were more significantly common in the intact membranes PTL group (30.8%) than in the control TL group (9.1%) (p = 0.0342; Table 1). A history of previous abortion was significantly more common in both the intact membrane PTL and PPROM groups than in the TL group (p = 0.0184 for both comparisons; Table 1). Heavy vaginal bleeding during current pregnancy was significantly more detectable in the PPROM group than in the TL group (p = 0.0465; Table 1). Also, only the PPROM group showed a significant more women with vaginal pH > 5 than in the TL group (p = 0.0104; Table 1).

A positive Whiff test was significantly more detected in the intact membranes PTL and PPROM groups than in the TL group (p = 0.0273 and 0.00345, respectively; Table 1). Other demographic characteristics, medical complications and speculum examination results were not significantly different among the PPROM, intact membranes PTL groups and TL group. Also, no significant difference was detected between the proportions of nulliparous, parous, primigravidas (data not shown) or those with previous PTB excluding primigravidas among the intact membranes PTL, PPROM and TL women (Table 1).

3.1.2. Microbiology

3.1.2.1. Identification of vaginal microorganisms and Gram stain of vaginal smear

Neisseria gonorrhoeae and *Mycoplasma genitalium* were not detectable; thus, they were not considered. The isolation of *Chlamydia trachomatis*, *Trichomonas vaginalis*, group B streptococci (GBS), Ureaplasma and *Mycoplasma hominis* were not significantly different among the three groups (data not shown). The heavy growth of vaginal organisms on sheep blood agar (SBA) was more significantly detected in the intact membranes PTL group (p = 0.0129), but not when lactobacilli were excluded, as shown in Table 2. Only coryneforms heavy growth was significantly associated with the intact membranes PTL and PPROM women than with TL women (p = 0.0077 for both comparisons; Table 2). The detection of Gram-negative bacilli was only significantly different between the intact membranes PTL and TL groups when considering the presence of any growth and not only the heavy growth (p = 0.0474; Table 2).

The heavy growth of other identified organisms on SBA and the lactobacilli growth on Rogosa agar were not significantly different among the three groups (data not shown). There was no significant difference among the intact membranes PTL, PPROM, and TL women in the presence of clue cells, vaginal grades, or bacterial vaginosis diagnosed by Amsel's criteria (data not shown).

3.1.2.2. Urinary tract infection

The urine samples' colony counts and all detected uropathogens were comparable among the three groups (data not shown). However, Gram-negative bacilli UTI was significantly more detected in the intact membranes PTL group than in the TL group (p = 0.0447; Table 2).

3.1.3. Odds ratio

The final multivariate model identified abortion history, heavy vaginal bleeding history, and elevated vaginal pH as the significant predictors of PPROM. However, age less than 20 years old, abortion history, the presence of heavy growth of vaginal organisms and any growth of Gram-negative bacilli were identified as the significant predictors of intact membranes PTL (Table 3). This final model has good p values for the goodness-of-fit tests done by Minitab 18 and is provided in Supplementary file 2, http://links.lww.com/JCMA/A45.[23]

Abortion history increased the risk for PPROM by seven-fold compared to women with no such history (OR = 7.68, 95% CI = 1.04-56.95; Table 3); however, this risk decreased to five-fold for intact membranes PTL for the same comparison (OR=5.76, 95% CI = 1.03-32.12; Table 3). Each one step increase in vaginal pH above 4.5 was associated with more than four-fold increase in the PPROM risk (OR = 4.71, 95% CI = 1.43-15.46; Table 3). The women with heavy vaginal bleeding had more than 14-fold increase in the risk of PPROM compared to women with no such history (OR = 14.69, 95% CI = 1.22-177.49; Table 3).

The presence of heavy growth of vaginal organisms was associated with about three times increase in the risk of intact membranes PTL compared with the group with no such growth (OR = 2.93, 95% CI = 1.06-8.07; Table 3), while any growth of Gram-negative bacilli was associated with more than two-fold increase in the risk of intact membranes PTL (OR = 2.75, 95% CI = 1.00-7.52; Table 3). Those less than 20 years old were subjected to more than five-fold increase in the risk of intact membranes PTL compared to women who were more than 20 years old (OR = 5.24, 95% CI = 1.40-19.63; Table 3).

Table 1

Comparison of demographic characters, pregnancy history, vaginal bleeding, and results of speculum examination among intact membranes PTL, PPROM, and TL women

Characteristic	TL (control)	Intact membranes PTL	PPROM	p	Adjusted p value (pairwise comparison with TL): Intact membranes PTL	Adjusted p value (pairwise comparison with TL): PPROM
Demographic characteristics (Number with characteristic/total number) (%)						
Age <20 years	4/44 (9.1)	16/52 (30.8)	5/18 (27.8)	0.024	0.0342	0.1590
Black race	2/45 (4.4)	4/53 (7.6)	5/18 (27.8)	0.024	0.6840	0.0507
Pregnancy history (Number with characteristic/total number) (%)						
History of PTB excluding primigravida	0/31 (0)	5/31 (16.1)	2/12 (16.7)	0.044	0.11	0.11
History of abortion excluding primigravida	2/31 (6.5)	11/31 (35.5)	5/12 (41.7)	0.006	0.0184	0.0184
Heavy vaginal bleeding	1/44 (2.3)	6/50 (12)	4/16 (25)	0.028	0.173	0.0465
Speculum examination (Number with characteristic/total number) (%)						
Other signs of infection (eg, erythema, eruptions)	1/41 (2.4)	4/47 (8.5)	4/18 (22.2)	0.042	0.366	0.0804
Vaginal pH ≤ 4.5	24/43 (55.8)	20/46 (43.5)	7/17 (41.2)			
Vaginal pH = 5	18/43 (41.9)	19/46 (41.3)	4/17 (23.5)	0.016	0.144	0.0104
Vaginal pH > 5	1/43 (2.3)	7/46 (15.2)	6/17 (35.3)			
Positive Whiff test	11/44 (25)	24/48 (50)	13/18 (72.2)	0.001	0.0273	0.00345

PPROM = preterm premature rupture of membranes; PTB = preterm birth; PTL = preterm labor; TL = term labor.

Table 2

Comparison of microbiological characters among intact membranes PTL, PPROM, and TL women

Characteristic (Number with characteristic/total number) (%)	TL (control)	Intact membranes PTL	PPROM	*p*	Adjusted *p* value (pairwise comparison with TL)	
					Intact membranes PTL	PPROM
Vaginal infection						
Heavy growth on SBA[a]	13/45 (28.9)	32/54 (59.3)	6/18 (33.3)	0.007	0.0129	0.767
Heavy growth on SBA except lactobacilli[a]	11/45 (24.4)	22/54 (40.7)	4/18 (22.2)	0.163	NA	
Coryneforms heavy growth[a,b]	0/45 (0)	9/54 (16.7)	4/18 (22.2)	0.002	0.0077	0.0077
Gram-negative bacilli growth at any streak	17/45 (37.78)	34/54 (63)	9/18 (50)	0.04	0.0474	0.409
Urinary tract infection characteristic						
Gram-negative bacilli UTI	0/45 (0)	7/54 (12.96)	2/18 (11.1)	0.021	0.0447	0.117

PPROM = preterm premature rupture of membranes; PTL = preterm labor; SBA = sheep blood agar; TL = term labor; UTI = urinary tract infection.

[a]The presence of characteristic growth at third or fourth streak.

[b]Coryneforms: *Brevibacterium* spp. (group B) in nine cases, *Arcanobacterium pyogenes*, *Acinetobacter calcoaceticus,* and *Corynebacterium minutissimum* each in one case and a mixed *Brevibacterium* spp. (group B) and *C. minutissimum* in one case.

Table 3

Logistic regression results for predictors of PPROM and intact membrane PTL women

Predictor	PPROM Unadjusted OR (95% CI)	*p*	Adjusted OR (95% CI)	Adjusted *p*	Intact membranes PTL Unadjusted OR (95% CI)	*p*	Adjusted OR (95% CI)	Adjusted *p*
Age < 20 years								
No	1 (Reference)	0.07	1 (Reference)	0.197	1 (Reference)	0.014	**1 (Reference)**	**0.014**
Yes	3.85 (0.9-16.5)		3.08 (0.56-17.02)		4.44 (1.36-14.53)		**5.24 (1.40-19.63)**	
Black race								
Non-black	1 (Reference)	0.02			1 (Reference)	0.53		
Black	8.27 (1.43-47.74)				1.76 (0.31-10.1)			
Abortion history								
No	1 (Reference)	0.01	**1 (Reference)**	**0.046**	1 (Reference)	0.01	**1 (Reference)**	**0.046**
Yes	10.36 (1.65-64.94)		**7.68 (1.04-56.95)**		7.98 (1.59-39.93)		**5.76 (1.03-32.12)**	
Heavy bleeding								
No	1 (Reference)	0.02	**1 (Reference)**	**0.035**	1 (Reference)	0.11	1 (Reference)	0.218
Yes	14.33 (1.46-140.53)		**14.69 (1.22-177.49)**		5.86(0.68-50.76)		4.56 (0.41-51.05)	
Signs of infection (eg, erythema, eruptions) other than vaginal discharge								
No	1 (Reference)	0.04			1 (Reference)	0.25		
Yes	11.43 (1.18-111.09)				3.72(0.4-34.72)			
Vaginal pH	4.4 (1.49-12.98)	0.007	**4.71 (1.43-15.46)**	**0.011**	2.86 (1.04-7.88)	0.04	2.74 (0.91-8.27)	0.073
Whiff test								
Negative	1 (Reference)	0.001			1 (Reference)	0.02		
Positive	7.8 (2.26-26.86)				3 (1.24-7.28)			
Mycoplasma hominis infection								
Negative	1 (Reference)	0.03			1 (Reference)	0.08		
Positive	12.57 (1.3-121.97)				6.55 (0.77-55.43)			
Heavy growth on SBA								
No	1 (Reference)	0.73	1 (Reference)	0.81	1 (Reference)	0.003	**1 (Reference)**	**0.038**
Yes	1.23 (0.38-3.98)		1.19 (0.29-4.87)		3.58 (1.54-8.32)		**2.93 (1.06-8.07)**	
Gram-negative bacilli growth at any streak								
No	1 (Reference)	0.38	1 (Reference)	0.39	1 (Reference)	0.014	**1 (Reference)**	**0.049**
Yes	1.65 (0.55-4.96)		1.81 (0.47-7.06)		2.8 (1.24-6.34)		**2.75 (1.00-7.52)**	
Baseline odds (exponentiated intercept)			0.00006				0.0017	

OR = odds ratio; PPROM = preterm premature rupture of membranes; PTL = preterm labor; SBA = sheep blood agar; TL = term labor.

3.2. The Detectability of different factors in SPTL, MPTL, and TL groups

3.2.1. Demographic characteristics, pregnancy history, medical complications, and speculum examination

No significant difference was detected among the three groups in the mean age, weight, or height. Also, other demographic characteristics were not significantly different among the three groups (data not shown).

Both previous PTB and previous abortion excluding primigravidas were significantly more common in the SPTL group compared to the TL group (p = 0.0198 and 0.00528, respectively; Table 4). However, the proportions of nulliparous, parous, or primigravidas women were not significantly different among the three groups.

Table 4

Comparison of demographic characters, pregnancy history, vaginal bleeding, and results of speculum examination among SPTL, MPTL and TL women

Characteristic	TL (control)	SPTL	MPTL	p	Adjusted p value (pairwise comparison with TL) SPTL	MPTL
Demographic characteristics (Number with characteristic/total number) (%)						
Age <20 years	4/44 (9.1)	9/30 (30)	6/20 (30)	0.034	0.088	0.088
Pregnancy history (Number with characteristic/total number) (%)						
One parous or more	31/45 (68.9)	16/31 (51.6)	9/21 (42.9)			
Nulliparous	0/45(0)	4/31 (12.9)	2/21 (9.5)	0.044	0.0525	0.0525
Primigravida	14/45 (31.1)	11/31 (35.5)	10/21 (47.6)			
History of PTB excluding primigravida	0/31 (0)	5/20 (25)	2/11 (18.2)	0.006	0.0198	0.0958
History of abortion excluding primigravida	2/31 (6.5)	9/20 (45)	3/11 (27.3)	0.004	0.00528	0.154
Heavy vaginal bleeding	1/44 (2.3)	4/27 (14.8)	4/20 (20)	0.025	0.0982	0.090
Speculum examination results (Number with characteristic/total number) (%)						
Vaginal pH ≤ 4.5	24/43 (55.8)	12/24 (50)	6/20 (30)			
Vaginal pH = 5	18/43 (41.9)	5/24 (20.8)	11/20 (55)	0.005	0.0109	0.0741
Vaginal pH > 5	1/43 (2.3)	7/24 (29.2)	3/20 (15)			
Whiff test positive	11/44 (25)	16/26 (61.5)	9/20 (45)	0.01	0.014	0.222

MPTL = mild preterm labor; PTB = preterm birth; SPTL = severe preterm labor; TL = term labor.

Heavy vaginal bleeding during the current pregnancy was not significantly different among the three groups (Table 4). Other medical complications were not significantly different among the three groups (data not shown).Vaginal pH >5 and positive Whiff test were significantly more detected in the SPTL group than in the TL group (p = 0.0109 and 0.014, respectively; Table 4). However, other speculum examination results were not significantly different among the three groups (data not shown).

3.2.2. Microbiology

3.2.2.1. Identification of vaginal microorganisms and Gram stain of vaginal smear

T vaginalis, GBS, Ureaplasma and *M. hominis* infections were not significantly associated with any of these groups (Table 5).

The heavy growth of vaginal organisms on SBA was only more significantly detected in the SPTL group than in the TL group (p = 0.0264; Table 5), but not when lactobacilli were excluded. Only coryneforms heavy growth was significantly more detectable in the SPTL and MPTL women than in TL women (p = 0.00125 and 0.0124, respectively; Table 5). The heavy growth of other identified organisms on SBA and lactobacilli growth on Rogosa agar were not significantly different among the three groups (data not shown). There was no significant difference among the SPTL, MPTL, and TL women in the presence of clue cells, vaginal grades, or bacterial vaginosis diagnosed by Amsel's criteria (data not shown).

3.2.2.2. Urinary tract infection

Only the urinary tract infections with ≥10^5 CFU/ml microbial count were significantly more common in the SPTL group than in the control group (TL) (p = 0.0366; Table 5). All identified uropathogens were not significantly different among the SPTL, MPTL, and TL groups (data not shown). When grouping the uropathogens, only Gram-negative bacilli UTI was significantly

Table 5

Comparison of microbiological characters among SPTL, MPTL, and TL women

Characteristic (Number with characteristic/total number) (%)	TL (control)	SPTL	MPTL	p	Adjusted p value (pairwise comparison with TL) SPTL	MPTL
Vaginal infection						
Mycoplasma hominis	1/45 (2.22)	3/31 (9.68)	4/21 (19.05)	0.049	0.42	0.097
Heavy growth on SBA[a]	13/45 (28.9)	19/31 (61.3)	8/21 (38.1)	0.019	0.0264	0.572
Heavy growth on SBA except lactobacilli[a]	11/45 (24.4)	12/31 (38.7)	6/21 (28.6)	0.3885	NA	
Coryneforms heavy growth[a,b]	0/45 (0)	8/31 (25.8)	4/21 (19.05)	0.0005	0.0013	0.0124
Urinary tract infection characteristic						
Urine bacterial count (CFU/ml)						
<10^3	42/45 (93.34)	24/31 (77.42)	17/21 (80.95)			
≥10^3 - <10^4	2/45 (4.44)	0/31 (0)	2/21 (9.52)	0.037	0.0366	0.342
≥10^4 - <10^5	1/45 (2.22)	3/31 (9.68)	1/21 (4.76)			
≥10^5	0/45 (0)	4/31 (12.9)	1/21 (4.76)			
Gram-negative bacilli UTI	0/45 (0)	4/31 (12.9)	3/21 (14.29)	0.017	0.0436	0.0436

MPTL = mild preterm labor; SBA = sheep blood agar; SPTL = severe preterm labor; TL = term labor; UTI = urinary tract infection.

[a]The presence of characteristic growth at third or fourth streak.

[b]Coryneforms: *Brevibacterium* spp. (group B) in eight cases, *Arcanobacterium pyogenes*, *Acinetobacter calcoaceticus,* and *Corynebacterium minutissimum* each in one case and a mixed *Brevibacterium* spp. (group B) and *Corynebacterium minutissimum* in one case.

more common in the SPTL and MPTL women than in TL women (p = 0.0436 for both comparisons; Table 5).

3.2.3. Odds ratios

The final multivariate model identified age less than 20 years old, abortion history, vaginal pH, and heavy growth of vaginal organisms as the significant predictors of SPTL and MPTL. Gompit link function was used as it gives the best p values for the goodness-of-fit tests done by Minitab 18 and is provided in Supplementary file 2, http://links.lww.com/JCMA/A45.[23]

Women less than 20 years old had more than three-fold increase in the risk of SPTL (or SPTL and MPTL) compared to women who were more than 20 years old (OR = 3.21, 95% CI = 1.59-6.44; Table 6). Those women with abortion history had also more than three-fold increase in the risk of SPTL (or SPTL and MPTL) compared to women with no such history (OR = 3.87, 95% CI = 1.73-8.64; Table 6).

As vaginal pH increases, women were more likely to have SPTL. Each one step increase in vaginal pH, above 4.5 was associated with 56% increase the odds of SPTL (or SPTL and MPTL) instead of TL (OR = 1.56, 95% CI = 1.05-2.33; Table 6). The presence of heavy growth of vaginal organisms was associated with about three times increase in the risk of SPTL (or SPTL and MPTL) compared to women with no such growth (OR = 2.78, 95% CI = 1.46-5.27; Table 6).

4. DISCUSSION

Several predictors were identified, in the final models, for the different studied subtypes of PTL. The association of age less than 20 years old with SPTL and intact membranes PTL was reported previously.[1,27] This may be due to the incomplete maternal physical growth and relative malnutrition.[28] On the other hand, PPROM was not associated with age less than 20 years old. This lack of association, between PPROM and age less than 20 years old, was described previously[1,5,8]

We detected an association between previous abortion and all PTL categories. This association has been described previously.[5,6,10] Women with a history of previous abortion may acquire the risk for subsequent PTB via the medical care they received, for example, antibiotic prophylaxis leading to alteration of vaginal microflora or cervical mechanical damage by uterine curettage in abortion procedure.[29] However, no association was recorded between PTB history and PPROM, although it was reported previously.[8] This may be attributed to the difference in the study design or the small number of women in the PPROM group who has a previous PTB (two cases).

In accordance with earlier studies, intense vaginal bleeding was associated with PPROM.[6,8] The association between vaginal bleeding and PTL may be due to the consequent thrombin production, which stimulates uterine contractions as well as proteolytic activity that can lead to PPROM.[4]

Elevated vaginal pH was significantly associated with all PTL forms, except the intact membranes PTL, similar to the results from previous studies. This was explained by the fact that elevated pH is a sign of inflammation or infection caused by abnormal vaginal flora with subsequent PTL.[30] This highlights the importance of vaginal pH measurement as one of the low cost, low technology, and safe protocols that are applicable to women in low-resource settings, for preliminary screening of PTL possibility and consequently reducing the rate of premature delivery.[31]

Healthy vaginal balanced microbiota (with lactobacilli or not) protect against ascending infections and prematurity[32]; therefore, disturbed vaginal flora, manifested by heavy growth of vaginal organisms on SBA, was associated with all PTL subtypes except PPROM. This lack of association between PPROM

Table 6

Adjusted odds ratios for predictors of SPTL and MPTL women

Predictor	Unadjusted OR (95% CI) for SPTL	p	Unadjusted OR (95% CI) for MPTL	p	Adjusted OR (95% CI)	Adjusted p
Age <20 years						
No	1 (Reference)	0.03	1 (Reference)	0.042	**1 (Reference)**	**0.001**
Yes	4.29 (1.18-15.58)		4.29 (1.05-17.45)		**3.21 (1.59-6.44)**	
Abortion history						
No	1 (Reference)	0.004	1 (Reference)	0.09	**1 (Reference)**	**0.001**
Yes	11.86 (2.21-63.78)		5.44 (0.77-38.3)		**3.87 (1.73-8.64)**	
Heavy bleeding						
No	1 (Reference)	0.08	1 (Reference)	0.04		
Yes	7.48 (0.79-70.88)		10.75 (1.12-103.56)			
Vaginal pH	3.3 (1.23-8.85)	0.02	4.25 (1.13-15.99)	0.03	**1.56 (1.05-2.33)**	**0.029**
Whiff test						
Negative	1 (Reference)	0.003	1 (Reference)	0.11		
Positive	4.8 (1.69-13.63)		2.45 (0.81-7.48)			
Mycoplasma hominis						
Negative	1 (Reference)	0.19	1 (Reference)	0.04		
Positive	4.71 (0.47-47.60)		10.35 (1.08-99.38)			
Heavy growth on SBA						
No	1 (Reference)	0.006	1 (Reference)	0.46	**1 (Reference)**	**0.002**
Yes	3.9 (1.48-10.27)		1.51 (0.51-4.51)		**2.78 (1.46-5.27)**	
Heavy lactobacilli growth on SBA						
No	1 (Reference)	0.03	1 (Reference)	0.71		
Yes	3.81(1.15-12.6)		1.33 (0.29-6.19)			
Gram-negative bacilli growth at any streak						
No	1 (Reference)	0.02	1 (Reference)	0.14		
Yes	2.99 (1.16-7.75)		2.20 (0.77-6.30)			

MPTL = mild preterm labor; OR = odds ratio; SBA = sheep blood agar; SPTL = severe preterm labor; TL = term labor.

and heavy growth of vaginal organisms on SBA may be due the small sample size of PPROM in our study or that anaerobic species were responsible for the vaginal dysbiosis, which could not be detected by aerobic growth on SBA.

In contrast to the protective effect of lactobacilli reported previously,[33,34] the detected vaginal lactobacilli in PTL group included *Lactobacillus iners* that can only be detected on blood agar. *L. iners* increases the risk of conversion from normal to abnormal flora with subsequent ascending infection and PTB.[32] It cannot synthesize lactic acid[31] and only a few strains can produce H_2O_2.[35] Consequently, these *Lactobacillus* species lacked the normal protective effects of lactobacilli.

The detection of Gram-negative bacilli was associated with intact membranes PTL when any growth (whether heavy or not) was used in statistical analysis. Gram-negative bacilli are important placental pathogens responsible for subclinical chorioamnionitis and PTB.[11,36]

Age of more than 35 years old was reported previously to be associated with PTL but not in our study.[6] This may be due to the low numbers of women of more than 35 years of age (six women) tested.

It is clear that prediction and management of the risk factors for PPROM and SPTL is highly required. SPTL is associated with more adverse outcome than MPTL,[10] also the maternal and neonatal morbidities of PPROM are significantly more pronounced when occur in SPTL case.[19] In our study, nearly half of the PPROM participants (seven out of 15 PPROM cases with accurately known gestational age) were with SPTL type and are susceptible to more adverse effects.

This study has a limitation of the small sample size in the tested subgroups, especially in PPROM group. This can negatively affect the statistic power of case collection. However, this study design has an acceptable statistic power for large ORs. In addition, this is only a pilot study to detect various factors that may be associated with the most risky forms of PTL (PPROM and SPTL). Other studies are required to further confirm this association, with each detected factor, on a larger population.

In conclusion, several factors were associated with the more dangerous PTL subtypes. Almost all these factors were related to infection with the exception of being less than 20 years old, which highlights the danger of infection as a main contributing factor for PTL. Infection control during abortion or labor is highly required to avoid its adverse effect on subsequent pregnancies. Women should be screened for the genitourinary infections during the prenatal and antenatal care, as many of these infections are asymptomatic. They should be treated with the suitable medications before fetomaternal damage occurs. Measuring vaginal pH can be used to predict women at risk for PTL before referral to a more advanced center for prenatal care, where the screening for these infections may not be possible in low-resource settings or when expensive kits are required (PCR for some infections).

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://doi.org/ 10.1097/JCMA.0000000000000243.

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