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Microbiological Result of the High Vaginal Swab in a Patient with Preterm Premature Rupture of the Membranes (PPROM) Versus Preterm Labor (PTL)

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Abstract

The complications of preterm birth cause approximately 70% of neonatal deaths and nearly half of all long-term neurological morbidity. In this study, we aim to report the findings of the laboratory investigations for high vaginal swabs (HVS) and their relation to PTL and PPROM. We performed a prospective cohort study of women presenting at The King Fahd Hospital of the University (KFHU) in Khobar, Kingdom of Saudi Arabia, with and without risk factors for preterm birth between December 2018 and December 2019. Women aged 15 to 40 years and satisfying inclusion criteria were included in the study. Finally, we included 117 women in the current study, with a total mean age of 30.5 ± 6.3 years and overall average gestational age, at delivery, of 33.8 ± 2.8 weeks. Out of those 117 women, 114 were diagnosed with having a PTL, while the other three had a PPROM. Among the 117 included women, 69 (58.9%) did not perform HVS, and only 48 women did. The findings were variable among PPROM and PTL groups. For PPROM, all of the three patients had a normal HSV with no detected organisms or pathological findings. Out of the 45 PTL patients who had HVS, we found a normal HSV (negative findings) in 39 patients (86.7%), the presence of yeasts in five patients (11.1%) and the presence of *Candida albicans* in one patient (2.2%). These findings indicate the urge to develop more advanced techniques for better detection of genital infections, and prevention of its complications.

Keywords: *High Vaginal Swab; PTL; PPROM; Preterm Labor*

Introduction

Premature labor is defined as every birth before 37 weeks gestational age [1]. The number of premature labor in the world is about 15 million globally. It is associated with several maldevelopments causing brain damages as attention deficit, autism, and hyperreactivity, and respiratory disorders [2-6]. The clinical and epidemiological characteristics are not fully comprehended [7-9]. Studies reported that diseases are more common in old, black, smoking women with socio-economic levels [7,9-14].

Genital infections have been frequently reported to cause preterm labor (PTL). Infections causing PML and preterm premature rupture of the membranes (PPROM) can reach the amniotic cavity through several pathways. The commonest one is by ascending from the maternal cervix or vagina. Other pathways include vertical transmission through the placenta, spread from the fallopian tubes, or mistakenly

when performing a surgical procedure. The mechanism of premature labor is that infections lead to activation of the innate immune system. Consequently, this leads to increased release of inflammatory mediators as interleukin 1β, interleukin 8 and tumor necrosis factor-α (TNF-α) which in turn stimulates the production of prostaglandins and other inflammatory mediators including degrading enzymes. Prostaglinds lead to increased uterine contractility while the degrading enzymes lead to the breakdown of the fetal extracellular matrix causing (PPROM) [15,16]. Furthermore, the fetal inflammatory response has been reported to be related to the onset of preterm labor, and long-term disabilities. A study reported that the prevalence of positive cultures was reported in 23% of 32 weeks' gestation premature infants [17-23].

The most prevalent microbial organisms in the amniotic fluid in premature and PPROM pregnancies are genital *Mycoplasma* spp. [24-27]. Moreover, lower genital tract infections found in the uterus, or amniotic fluid have been reported to be of low virulence. Therefore, the cause of premature labor is the chronicity of infection as studies suggest that bacteria alone cannot cause premature labor as positive cultures were detected in 80% of pregnancies before having an intact membrane and normal labor. However, together with the inflammatory response, bacterial infection can easily cause premature labor [15,28].

Several organisms have been associated with premature labor. Studies report that vaginal cultures differ with different ethnicities which indicates the need for more and more investigations for further mapping of the prevalence of these organisms, and therefore put the management techniques [29-32]. In this study, we aim to report the findings of the laboratory investigations for high vaginal swabs (HVS) and their relation to PTL and PPROM.

Methods

Study design and setting

We performed a prospective cohort study of women presenting at The King Fahd Hospital of the University (KFHU) in Khobar, Kingdom of Saudi Arabia, with and without risk factors for preterm birth between December 2018 and December 2019.

Study population and procedures

Women aged 15 to 40 years were included in the study if they have a singleton pregnancy, preterm pregnancy (defined as pregnancy at gestational age 28 - 37 weeks), PPROM, chorioamnionitis, or a previous history of preterm birth. We excluded women with multiple gestations, severe anemia, alcohol consumption, tobacco smoking, or a psychiatric condition precluding informed consent.

The HVS were collected from all enrolled women prior to vaginal examination. Vaginal samples obtained using sterile cotton-tipped swabs were sent to the laboratory in the transport medium. In the laboratory, the samples were aliquoted into two separate tubes: one for direct wet-mount microscopic examination and the other for Gram staining and subsequent inoculation onto blood and MacConkey agar plates. An examination of wet preparations was performed to detect the presence of yeasts. Gram staining was used to detect the presence of yeasts, inflammatory cells, and possible pathogenic microorganisms and to diagnose bacterial vaginosis by observing the "clue cells" [33].

Informed consent and ethical considerations

No identifying information of any respondent was published and all collected data were exclusively used for statistical analysis. Before commencement, the study protocol was cleared by the institutional review board and the ethics committee. Consent was obtained prior to starting the survey and after the appropriate explanation.

Statistical analysis

Mean and standard deviation (SD) were used to represent continuous variables while we used frequencies and percentages to represent categorical variables. The skewness and Kurtosis tests were used for testing the normal distribution of continuous variables. Chi^2 test

(or Fisher's exact test, as appropriate) was used for categorical data while the t-test was used for continuous variables normally distributed and the or Mann-Whitney U test was used for continuous variables not normally distributed. All analysis was performed with IBM SPSS Statistics version 26.0. The statistical significance was considered when the P-value was < 0.05.

Results

Baseline characteristics of the included patients

Finally, we included 117 women in the current study, with a total mean age of 30.5 ± 6.3 years and overall average gestational age, at delivery, of 33.8 ± 2.8 weeks. Out of those 117 women, 114 were diagnosed with having a PTL, while the other three had a PPROM. Most of the included women were of Saudi nationality (87.2%), followed by Yemeni (4.3%) and Filipino (3.4%) nationalities, respectively. All patients with PPROM did not have diabetes Mellitus or hypertension. In the same context, most of the patients with PTL did not have either co-morbidities (86.3%); nevertheless, eight women had gestational diabetes mellitus, four had diabetes mellitus, one with both diseases, one with gestational diabetes mellitus, one with gestational hypertension, and one with hypertension (Table 1).

Both patient groups were matched in mean age (PPROM: 26.0 ± 3.6; PTL: 30.6 ± 6.3; P-value = 0.209) and body mass index (BMI) (PPROM: 25.9 ± 4.7; PTL: 30.3 ± 6.3; P-value = 0.335), with no statistically significant differneces. Hoewever, there was a statistically significant difference bweteen the two groups in the average gestational age at delivery (PPROM: 29.0 ± 5.3; PTL: 33.9 ± 2.7; P-value = 0.335) (Table 1).

N		PPROM		Preterm labor		Total		P-value
		%	N	%	N	%		
Age; Mean ± SD		26.0 ± 3.6		30.6 ± 6.3		30.5 ± 6.3		0.209
Gestational age at delivery (weeks); Mean ± SD		29.0 ± 5.3		33.9 ± 2.7		33.8 ± 2.8		0.022*
BMI; Mean ± SD		25.9 ± 4.7		30.3 ± 6.3		30.2 ± 6.3		0.335
Nationality	Egyptian	0	0.0%	2	1.8%	2	1.7%	1.000
	Jordanian	0	0.0%	2	1.8%	2	1.7%	
	Filipino	0	0.0%	4	3.5%	4	3.4%	
	Saudi	3	100.0%	99	86.8%	102	87.2%	
	Sudanese	0	0.0%	1	0.9%	1	0.9%	
	Syrian	0	0.0%	1	0.9%	1	0.9%	
	Yemeni	0	0.0%	5	4.4%	5	4.3%	
DM and/or Hypertension (HTN)	DM	0	0.0%	4	3.5%	4	3.4%	1.000
	DM and HTN	0	0.0%	1	0.9%	1	0.9%	
	GDM	0	0.0%	8	7.0%	8	6.8%	
	Gestational DM	0	0.0%	1	0.9%	1	0.9%	
	Gestational HTN	0	0.0%	1	0.9%	1	0.9%	
	HTN	0	0.0%	1	0.9%	1	0.9%	
	No	3	100.0%	98	86.0%	101	86.3%	

Table 1: *Baseline characteristics of the included patients.*
*BMI: Body Mass Index; DM: Diabetes Mellitus; GDM: Gestational Diabetes Mellitus; PPROM: Preterm Premature Rupture of the Membranes; SD: Standard Deviation; *: Statistically Significant.*

Comparison of microbiological findings

Among the 117 included women, 69 (58.9%) did not perform HVS, and only 48 women did. The findings were variable among PPROM and PTL groups. For PPROM, all of the three patients had a normal HSV with no detected organisms or pathological findings. Out of the 45 PTL patients who had HVS, we found a normal HSV (negative findings) in 39 patients (86.7%), the presence of yeasts in five patients (11.1%), and the presence of *Candida albicans* in one patient (2.2%) (Figure 1).

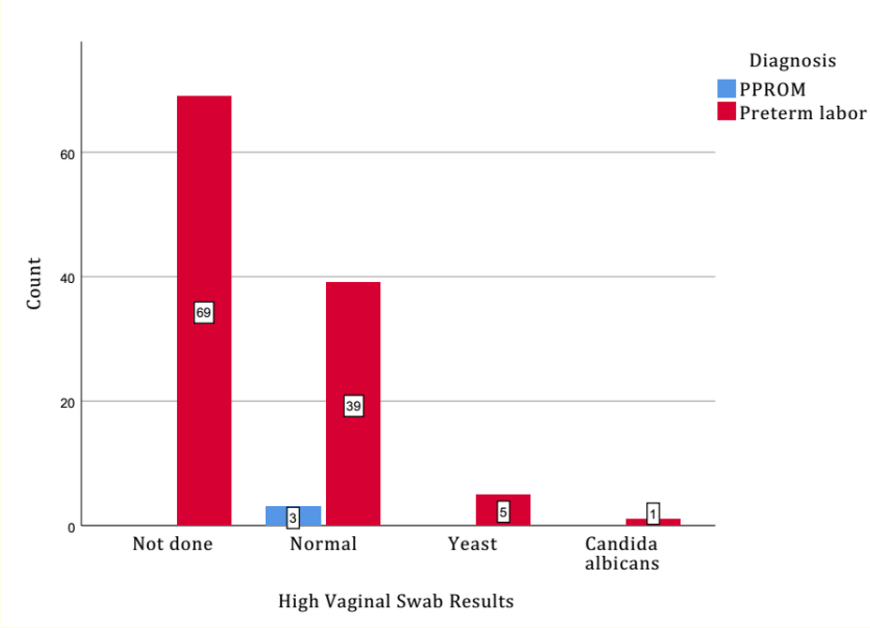

Figure 1: *Comparison of microbiological finding in both PPROM and preterm labor.*

Discussion

Intrauterine infections account for 25 - 40% of premature labor [15,34]. However, underestimation of this rate must be considered due to the non-availability of highly sensitive and specific culture techniques [35]. Several considerations have been given to the relationship between the microbiology of the vaginal organisms and the incidence of preterm birth (PTB) [36-39]. Despite being common, an acceptable explanation for the pathogenesis of the infection causing PTB is still not yet declared. As the vagina contains several normal florae and many cases may be asymptomatic, it is difficult to define the abnormality of the vaginal microbiology. As mentioned before, the detection and culturing of the vaginal microbes can be difficult not only due to the lack of techniques but also due to external factors and the different ethnicities of patients [40]. Breugelmans., *et al.* found no significant association between PTB and the presence of abnormal vaginal microbiology [38]. Therefore, despite being a risk factor for PTB, the mechanism of bacterial vaginosis (BV) to cause PTB is still unknown [41]. Cho., *et al.* reported no significant correlation between BV and PTB [42]. A possible explanation is that BV is usually reported in the first trimester and when pregnancy advances, the prevalence decreases. Moreover, differences in the diagnostic criteria of BV may play a significant role [41].

In this study, 39 PTL and three PPROM women had normal HVS cultures. Moreover, five cultures showed yeast infection, and only one showed *Candida albicans*. Fungal infections were also reported by previously published reports [24-27]. Cho., *et al.* reported a prevalence

rate of 4% of monilia [42]. Nakubulwa., *et al*. found a statistical significance in the association between PPROM and candidiasis [43]. However, bacterial infection accounted for the highest prevalence in almost all studies. Moreover, opportunistic viral infections as varicella pneumonia and severe acute respiratory syndrome viruses have been also reported to cause PTB in patients with severe illnesses, or low immunity [44,45]. However, there is poor evidence on this correlation as reports showed that the presence of viral infection does not necessarily end up with PTL or PPROM [46,47]. Overall, the significance of microbial infection and PTL or PPROM is not always understandable. Only some infections have been reported to have associations that can vary from strong to poor [48,49]. Trichomoniasis, syphilis, and gonorrhea infections [50,51] are related to premature delivery while *U. urealyticum*, group B *Streptococcus* and *M. hominus* [48,49] are not. Some organisms play a significant role when other risk factors are present. For example, *Chlamydia* infection can cause it only with a maternal immune response [52].

A variety of reports reported multiple cutoffs for the gestational age have been used to differentiate between spontaneous abortion and PTL. In the USA the cutoff has been reported to 13%, and 5 - 9% in other developed countries [9,53]. The mean gestational age in our study was 29 (± 5.3) weeks for PPROM and 33.9 (± 2.7) weeks for PTL with a statistical significance between the two groups (P = 0.022). The mean total gestational ages for all of our included patients is consistent with the results of other studies in Libya [54], Iran [55], India [56], Egypt [57] and South Africa [58]. The explanation for the high PTL in pregnant women at low gestational ages is the more freedom of the fetus while after 37 weeks of gestation, the fetus becomes more fixed [59]. Moreover, high prevalence rates of intrauterine infections have been associated with early gestational ages when PTL occurred [60,61]. Moreover, low BMI is reported to have a positive correlation with PTB [62]. In our study, the mean BMI was 30.2 (± 6.3). Hendler., *et al*. results showed that women with BMI < 19 had the highest rate of PTB [63]. Moreover, the mean maternal age was 30.5 (± 6.3) years which is consistent with the results of Freitas e*t al*., however, we found no significance between the PTL and PPROM groups [62]. Gestational DM was the most prevalent comorbidity, occuring in eight patients which is similar to the results by Addisu., *et al* [64].

Conclusion

In conclusion, gestational age can be used for predicting PTB due to its significant value. Although BMI and maternal ages can be used for prediction, and prevention of PTL and PPROM, we found no significant difference between the two groups. Our microbiological findings indicate the urge to develop more advanced, highly sensitive techniques for better detection of genital infections and prevention of its complications.

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Conflict of Interest

The authors declare no conflict of interest.

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