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ORIGINAL ARTICLE

Preterm Labour: An insights into Vaginal Infections

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ABSTRACT

Background: Preterm births result from preterm labour. The chief causes of new-born neurological morbidity and transience are prematurity and low birth weight. Vaginal infections are considered as a higher risk factor for pre term labour. Preterm labour is less common when proper antenatal examination, screening for lower urogenital tract infections, and early treatment are carried out.

Methods: Group B streptococcus (GBS), Urea plasma urealyticum, Mycoplasma genitalium, Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, bacterial vaginosis, herpes simplex virus (HSV) I and II, were all examined in vaginal swab samples from A control group of 81 expectant mothers underwent a GBS test. Tests for the antimicrobial propensities of GBS, U. urealyticum and M. hominis were conducted.

Results: 8.70% of PTL-WO, 16.33% of S-PTB, 11.70% of M-PTB, and 17.29% of the control group had GBS. 13.04% of PTL-WO, 18.37% of S-PTB, and 17.65% of M-PTB had M. hominis. U. urealyticum was detected by PCR and culture in 52.17 percent of PTL-WO, 48.98 percent of S-PTB, and 55.88 percent of M-PTB. 17.39% of PTL-WO patients, 4.08% of S-PTB patients, and 5.88% of M-PTB patients had C. trachomatis. Monilia was found in 4.3% of PTL-WO, 6.12% of S-PTB, and 5.88% of M-PTB. This examination failed to discover N. gonorrhoeae, M. genitalium, HSV I, T. vaginalis, or T. pallidum; no additional bacteria or viruses were detected either.

Conclusions: Lower urogenital tract infections in pregnant women should be tested for, especially in high-risk situations. When genitourinary infections are detected early and treated fast, prematurity-related infant morbidity and mortality are decreased, and preterm labour is less common.

Keywords: Preterm labour, Urinary tract infection, vaginal infections

INTRODUCTION

Preterm labour [PTL] is defined as onset of regular uterine contractions associated with cervical changes between gestational age of 28 to 37 completed weeks. PTL is suspected when a woman presents with frequent uterine contractions occurring at least once every 5–8 minutes, cervical dilation more than 2 cm and cervical effacement (≥50%) with gestational age between 28 to 37 weeks. Preterm Birth (PTB) issues are responsible for > 50% of all occurrences of long-term neurological morbidity and around 70% of all neonatal deaths (Souza et al., 2020). The most frequent subtypes of PTB include preterm premature rupture of the membranes (PPROM), medically induced preterm birth (M-PTB), and spontaneous preterm labour (S-PTB) resulting in preterm labour (PTL), PTL without preterm delivery (PTL-WO) which can all be classified according to the clinical presentation (Campbell, 2018). Although some of the risk factors for S-PTB have been identified, not all of them have, and the health care scheme is inept to effectively aim and cope momentous risk factors (Oliver and Lamont, 2013). As a result, treating S-PTB patients is challenging. It has been proposed that the existence of an intrauterine infection is the most dangerous factor for pregnancy complications, including preterm membrane rupture, premature labour, premature birth, and perinatal infections. The cervix and vagina, transplacental infection, regressive seeding from the peritoneal cavity through the fallopian tubes, retrograde seeding from the peritoneal cavity through the fallopian tubes, and accidental introduction during invasive procedures like amniocentesis, percutaneous foetal blood sampling, and chorionic villus sampling are all possible routes for germs to enter the amniotic cavity (Zahaby, 2012). Most of the research on the identification of infection in patients with PTL has been on the microbial invasion of the normally sterile amniotic cavity (DiDiulio, 2012). This is because an infection is most likely to spread to the amniotic cavity. This is because of the amniotic cavity typically being sterile. As a result, the ability to separate any organism from amniotic fluid demonstrates the existence of microorganisms in the uterine environment (Collado et al., 2016). The ascending channel is unquestionably the most frequent route for an infection to spread within the uterine cavity.

While some infections have been found to have a consistent link to preterm delivery, others have only been shown to have a chance link to a specific organism's colonisation of the mother's vaginal canal (Wassenaar and Panigrahi, 2014). Even if some diseases are related to premature birth, this is still the case. Reduction in the prevalence of preterm labour (PTL) in women who already have the disease, it is hoped that recurrent vaginal infections would be identified and treated (Blostein et al., 2017).

Some of the bacteria that are connected to PTL but are not the reason include Gardnerella vaginalis, Trichomonas vaginalis, Ureaplasma urealyticum, Treponema pallidum, Mycoplasma hominis, Neisseria gonorrhoeae, group B streptococcus (GBS), peptostreptococci, Chlamydia trachomatis, and Bacteroides spp (Choi et al., 2012). It is challenging to determine the degree of the contributory relationship among infection and S-PTB because rates of maternal genital microbe colonisation vary depending on gestational age, regional variance, race, and researchers (Gimenez et al., 2016). Furthermore, it is unknown how or when bacteria enter the uterus, nor is it known whether PTL is caused by any other (yet inexplicable) infections caused by bacteria, protozoans, or viruses.

The prevalence of vaginal bacteria that were isolated from females who had PTL-WO and S-PTB was assessed in this study. Additionally, an analysis of the bacteria' antibiotic susceptibilities was done. We searched for microorganisms that might possibly be PTL risk factors as the last phase in our research.

METHODOLOGY

Study Sample: The study included 106 pregnant ladies who had PTL, and the control group included 81 pregnant ladies who had no previous record of PTL or preterm delivery and who received routine prenatal care. All the participants in the study were admitted to the Zanana DHQ Teaching Hospital Dera Ismail Khan between September 2021 and April 2022. The patients were classified into one of three categories, based on the severity of the difficulties they experienced during their pregnancies: PTL-WO (with a sample size of 23), S-PTB (with a sample size of 49), and M-PTB (with a sample size of 34). PTL with risk factors such as spontaneous anomalies of conception, serious maternal disease,

rupture of membranes, multiple gestations, cervical incompetency, malformations of the foetus, hydramnios, uterine anomalies, overdistended uterus, foetal death, gestational hypertension, gestational diabetes mellitus, retained intrauterine device, pre-eclampsia, and active systemic infection were risk factors for M-PTB. The Institute gave its stamp of approval to the study's protocol.

Genital Microbes

GBS culture and antimicrobial susceptibility test: Pregnant women's vagina, anorectum, and urethral orifices were swabbed, and samples were then put in discerning Todd-Hewitt broth (S-THB, new Granada medium for 18 to 24 hours at 35 C in a 5% CO_2 atmosphere. Colonies that showed growth turbidity in S-THB and/or an orange colour on new Granada tube or plate medium were subculture on 5% sheep blood agar for identification with the Streptex group B Streptococcus reagent. Bacterial susceptibility to antibiotics i.e., (erythromycin, clindamycin, chloramphenicol, penicillin, levofloxacin, vancomycin, and ceftriaxone) by using the MicroScan® MICroSTREP plus panel. Using a Renok hydrator/inoculator, 115 µL of Mueller-Hinton broth containing 3% lysed horse blood was poured into each well to inoculate the panel. The panels were inoculated with 0.5 McFarland standard bacterial suspension and incubated at 35 C in ambient air for 20 to 24 hours before the readings were taken using the MicroScan® WalkAway System (Uh et al., 2009).

M. hominis and U. urealyticum antimicrobial susceptibility tests: To conduct the Mycoplasma IST 2 (bioMérieux) test, a vaginal swab sample was collected from each pregnant woman. The Mycoplasma IST 2 test comes with strips that show whether M. hominis and U. urealyticum are present or absent, as well as additional data on antibiotic susceptibility to doxycycline, tetracycline, erythromycin, azithromycin, clarithromycin, josamycin, ofloxacin, ciprofloxacin, and pristinamycin. In addition, the strips show whether M. hominis is present or absent. One strip was sent to the clinical microbiology lab in R1 tubes, where it was immediately analysed for the bacteria's identification and whether they were resistant to antibiotics. It was immediately combined by vortexing the two of them, and then 3 mL of R1 was used to rehydrate the lyophilized growth medium R2. After that, the rehydrated R2 growth medium was used to inoculate a Mycoplasma IST strip with 22 wells. The volume of liquid utilised in each well was 55 litres, and the top layer consisted of two drops of mineral oil. To investigate the distinct colony morphology, 0.1 millilitres of the R2 positive tube's contents were transferred to A7 Mycoplasma agar plates (BioMérieux) and incubated for 24 to 48 hours at 37 degrees Celsius in an atmosphere containing 5% carbon dioxide while the colour of the plates was monitored [11]. M. hominis and U. urealyticum were found in wells 1 through 5 and their density was assessed to be 104 CFUs per well. When the colony count of each organism reached 104 CFU/mL, the antimicrobial susceptibility test was performed on the samples (Lee et al., 2016).

Genital multiplex PCR: Samples for multiplex PCR were taken by swabbing the posterior vaginal fornix with a cytobrush. This was done to acquire the DNA. The PCR buffer was used to wash the cotton swab. Using the Seeplex ® STD6B ACE Detection kit, it was possible to concurrently identify M. hominis, T. vaginalis, Mycoplasma genitalium, C. trachomatis, U. urealyticum, T. pallidum, herpes simplex virus I&II, and N. gonorrhoeae (Seegene, Seoul, Korea). After being vortexed, the sample that was swabbed was centrifuged at 3,000 rpm for twenty minutes, and then it was incubated at 56 degrees Celsius for more than three hours. After being resuspended in a solution containing sodium acetate, ethanol, and phenol-chloroform, the pellet was placed in an incubator on ice for a period of thirty minutes. To get the DNA, centrifugation at room temperature for five minutes at 14,000 rpm yielded a pellet that was subsequently frozen at -20 degrees Celsius. After being washed with 70% ethanol and resuspended in sterile distilled water, the extracted DNA was then prepared for use in multiplex PCR. Multiplex PCR was performed by following the methodology presented by Cho et al. (2013).

Culturing, Wet mounting, and Gram staining vaginal specimens: The vaginal samples were collected using sterile cotton-tipped swabs, and once they were brought back to the lab, they were separated into two tubes: one was used for Gram staining, and the other was utilised to inoculate blood and MacConkey agar plates after direct wetmount microscopy analysis. Gram staining was utilised to find inflammatory cells, yeasts, and potentially pathogens, and to identify "clue cells" that would indicate the presence of bacterial vaginosis (BV) (Zaki et al., 2010).

Statistical Analysis: In PASW Statistics 18, the Fisher's exact test was used to conduct the statistical analysis, and p values of 0.05 were considered statistically significant.

RESULTS

The colonisation rates of GBS were 8.70% in the PTL-WO group, 16.33% in the S-PTB group, 11.70% in the M-PTB group, and 17.29% in the control group. Even though the rate of GBS colonisation in the S-PTB group was more than triple that of the PTL-WO group, there was statistically suggestive difference between the two groups. The rate of GBS colonisation in the control group was substantially higher than in the sick groups. M. hominis detection was 13.04% frequent in the PTL-WO group, 18.37% often in the S-PTB group, and 17.65% frequent in the M-PTB group. Even though M. hominis was discovered more frequently in the S-PTB group than in the PTL-WO group, there was a statistically significant difference between the S-PTB and PTL-WO groups. The PTL-WO group had a 53.8% detection rate, whereas the S-PTB group had a 60.9% detection rate and the M-PTB group had a 74.2% detection rate. Even though U. urealyticum was discovered more frequently in the S-PTB group than in the PTL-WO group, there was a statistically enticing difference between the two groups. The rate of U. urealyticum detection by PCR and culture was 52.17% in the PTL-WO group, 48.98% in the S-PTB group, and 55.88% in the M-PTB group. C. trachomatis was detected 17.39% of the time in the PTL-WO group, 4.08% of the time in the S-PTB group, and 5.88% in the M-PTB group. 4.3% of those in the PTL-WO group, 6.12% of those in the S-PTB group, and 5.88% of those in the M-PTB group were positive for monilia. This examination failed to detect HSV I, M. genitalium, T. vaginalis, N. gonorrhoeae, or T. pallidum; in fact, none of the other bacteria or viruses under investigation were found either (Table 1).

Table 1. Prevalence of lower genital tract microbes in pregnant women

Causal Organisms	CTRL Group (81)	PTL-WO (23)	Pregnancy Problems		Total (106)
			PTB		
			S-PTB (49)	M-PTB (34)	
S. agalactiae	14	2	8	4	14
M. hominis		3	9	6	18
U. urealyticum		12	24	19	55
C. trachomatis		4	2	2	8
HSV II		0	3	1	4
Monilia		1	3	2	6
Clue Cells		1	0	0	1

Antimicrobic susceptibility examination: The resistance rates of Clindamycin (6.8, 0, 17.8 and 35.9%), erythromycin (6.8, 90, 15.9 and 0%), chloramphenicol (0, 95, 0 and 0%), and levofloxacin (0, 100, 0 and 35.4%) respectively, in GBS isolates. All GBS isolates were resistant to ceftriaxone, vancomycin, and penicillin. The M. hominis cultures obtained from different patients were responsive to josamycin and pristinamycin but resistant to erythromycin, tetracycline, ciprofloxacin, ofloxacin, clarithromycin, and azithromycin. One of the two patients' M. hominis cultures was doxycycline susceptible. For U. urealyticum isolates, the corresponding resistance rates were such as ciprofloxacin (78.5, 76.1 and 92.4%), ofloxacin (76.5, 75.4 and 62.3%), tetracycline

(13.2, 14.1 and 12.8%), azithromycin (10.5, 11.9 and 11.8%), erythromycin (11.8, 7.6 and 18.7%), clarithromycin (9.8, 7.6 and 6.4), and doxycycline (0, 7.2 and 11.5%). Most of isolate of U. urealyticum were responsive to pristinamycin and josamycin (Figure 2 A, B, C, D).

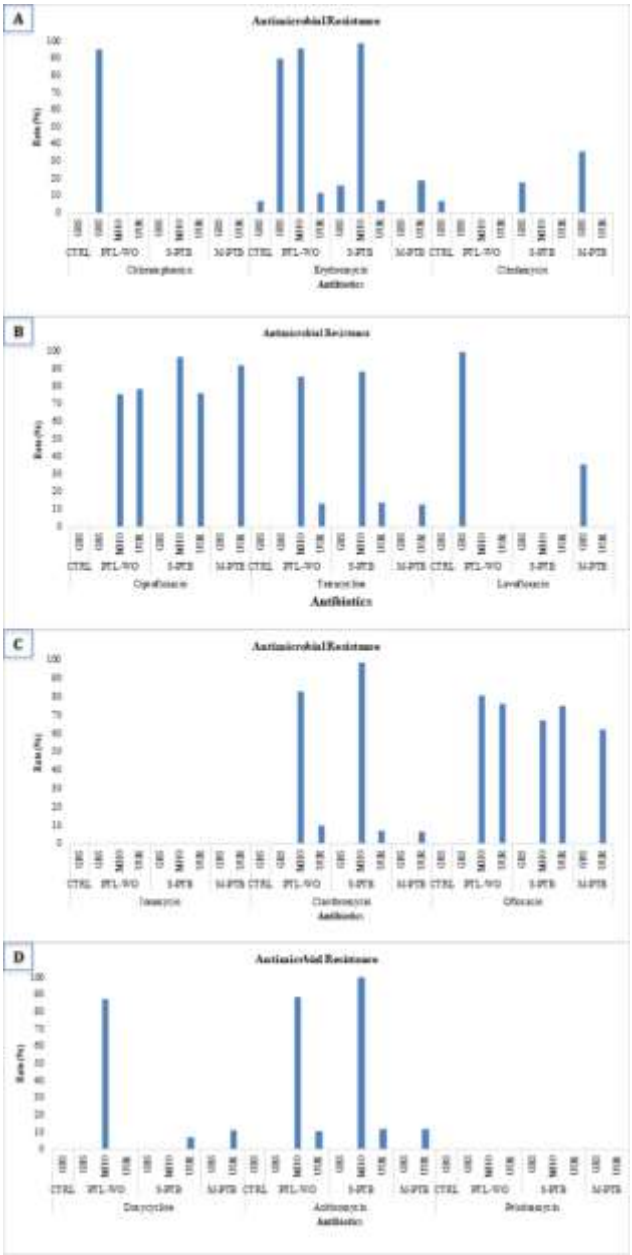

Figure 1: (A, B, C, D). GBS, Mycoplasma hominis, and Ureaplasma urealyticum antimicrobial resistance (%) in pregnant women

DISCUSSION

A significant amount of focus has been placed on the association between PTL and shifts in the vaginal flora that occur during pregnancy (Lamont et al., 2011). S-PTB is a syndrome that affects a relatively large population, although the elements that contribute to its development remain unknown (Leonard et al., 2019).

It may be quite difficult to identify abnormal bacteria in a pregnant woman's vaginal tract because of the hormonal changes that occur during pregnancy. The researchers believe that the morbific role of microbes in the vagina as hazard factors for S-PTB varies because lower genital region bacteria are more commonly isolated from women with intrauterine diseases. This is because patients with intrauterine infections tend to have lower genital tract bacteria. There is a possibility that the colonisation rate of bacteria in the vagina is pretentious not only by methodological elements such as the approach of detection and the sample site, but also by inner and outside factors that are specific to everyone (Olsen et al., 2018). There may be variations in the configuration of the usual vaginal microbes based on a person's race or ethnicity. The absenteeism of vaginal lactobacilli was a clearer indicator of S-PTB at 33 calendar weeks of pregnancy than the incidence of M. hominis, although its sensitivity and encouraging predictive amount were only slightly higher than those of M. hominis, as stated by Usui et al. (2002). According to Breugelmans et al. (2010), there is no definitive link between irregular vaginal microbes and S-PTB, and the danger of S-PTB amplified when Ureaplasma spp. are present in an abnormal vaginal flora. However, there is no definitive link between abnormal vaginal flora and S-PTB.

Although BV has been regarded as a risk factor for S-PTB, researchers have been unable to definitively establish a connection between the two. The fact that clue cells were only found in 4.35 percent of the S-PTB group during this investigation lends credence to the hypothesis that there is no link between BV and S-PTB. Both the fact that BV is typically diagnosed in the first trimester while the prevalence declines in both the second and third trimesters and the possibility that the relationship between BV and S-PTB varies depending on the diagnostic criteria for the detection of BV could provide an explanation for our findings (Donati et al., 2010).

Patients who have PPROM and those who have PTL-WO are more likely to have the organisms U. urealyticum and M. hominis detected from both the placental membranes and the amniotic fluid (Thi Trung Thu et al., 2018). Both types of organisms can initiate the production of prostaglandins, which results in the formation of S-PTB. There is a significant amount of debate about the question of whether genital mycoplasmas have deleterious effects or are essentially harmless invaders of the vaginal canal. According to the common belief, genital mycoplasmas are frequently discovered in the vaginal microbiota of females. However, in contrast to their presence in the upper genital tract, the presence of genital mycoplasmas in the lower genital tract is not associated with an increased risk of syphilis or pyogenic urethritis (Sarier and Kukul, 2019).

GBS has evolved as a significant pathogen that can cause a diverse bacterial infection in pregnant ladies, people who are not pregnant, and elderly patients. GBS is part of the natural flora that can be identified in the vagina of most pregnant women. GBS is deemed a hazard factor for S-PTB since of its relation to symptomless bacteriuria, as stated by Garland et al. (2000). These researchers came to this conclusion after doing research. On the other hand, when it exclusively colonises the lower genital tract, it is not thought to stimulate preterm labour. The relative risk (RR) of GBS in S-PTB was much lower in this study, which indicates that GBS was not a hazard factor in and of itself for the advancement of S-PTB from PTL-WO.

Even though N. gonorrhoeae and C. trachomatis are rarely associated with uterine or foetal infection prior to membrane rupture, it is possible for both organisms to have a deleterious impact on pregnancy, increasing the likelihood of preterm labour. Studies have shown that N. gonorrhoeae affects one percent of women who are pregnant and, if left untreated, increases the risk of preterm birth by two to five percent (Heumann et al., 2017). During our examination, the polymerase chain reaction (PCR) did not turn up any evidence of HSV I, M. genitalium, T. vaginalis, or T. pallidum. In contrast, the prevalence of Chlamydia trachomatis and Neisseria gonorrhoeae was determined to be 7.5% and 0%, respectively.

Women who are pregnant and have PPROM and take antibiotics have healthier babies and longer pregnancies, as well as lower rates of clinical chorioamnionitis and newborn sepsis. It

has not been demonstrated that the benefit is realised by patients who have PTL-WO and undamaged membranes. A significant amount of research is required to understand why some ladies develop an mounting intrauterine contagion while others do not, as well as which strategies can lessen the harmful effects of systemic foetal soreness. In addition, this type of research is required to identify potential treatments for systemic foetal inflammation. An extensive randomised study indicated that treating pregnant women with erythromycin for U. urealyticum vaginal colonisation had no benefit for lowering the incidence of S-PTB (Choi et al., 2012). This was the conclusion reached by the researchers. According to the results of this study, the level of erythromycin resistance in U. urealyticum was lower, whereas the level of ciprofloxacin resistance was significantly higher.

CONCLUSION

Our results verified that GBS and genital mycoplasmas in the lower genital region were not hazard factors for the formation of S-PTB from PTL-WO, even though the exposure frequencies of these bacteria in S-PTB were greater than those in PTL-WO. Even though these bacteria were detected at higher rates in S-PTB than in PTL-WO, this was the case. Rather than only the presence of the bacteria, the host's reaction to the incidence of GBS and genital mycoplasmas may change, increasing the likelihood of S-PTB. The presence of the microorganisms themselves contrasts with this.

One of the study's limitations was the lesser number of PTL affected patients that were registered in it. Additionally, only GBS colonisation was examined in the control group, which had neither PTL nor PTB. To shed insight on the pathogenic involvement of these microbes in PTL-WO and S-PTB, additional in-depth research on the colonisation rates of GBS, M. hominis, and U. urealyticum in healthy preterm women who have not yet given birth or gone into labour is required.

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