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CLINICAL ARTICLE

Maternal risk factors for abnormal vaginal flora during pregnancy

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ABSTRACT

Objective: To determine the prevalence of abnormal vaginal flora during pregnancy and associated maternal risk factors. *Methods:* A retrospective study was undertaken of cervicovaginal smears performed on pregnant women at a center in Turin, Italy, between 2000 and 2010. Patients were divided into three groups: women with symptoms of genital infections (G1), asymptomatic women at risk of preterm birth (G2), and asymptomatic women with no risk (G3). Logistic regression models identified variables associated with microorganisms. *Results:* Among 11 219 samples, 4913 (43.8%) were positive, of which 3783 (77.0%) were positive for a single microorganism. Multivariate analysis for G1 showed positive associations between multiple sexual partners and bacterial vaginosis/*Ureaplasma urealyticum*, and multiparity with preterm birth and *U. urealyticum* ($P < 0.05$ for all). In G2, there were significant associations between multiparity with preterm birth and bacterial vaginosis/aerobic vaginitis, and North African origin and bacterial vaginosis/*U. urealyticum* ($P < 0.05$ for all). In G3, there were associations between little education (<8 years) and bacterial vaginosis/*U. urealyticum*, multiple sexual partners and bacterial vaginosis/*U. urealyticum*, and bacterial vaginosis and Eastern European origin and not being married ($P < 0.05$ for all). *Conclusion:* Positive cervicovaginal smears were associated with a particular profile. Testing could be advisable for symptomatic women at any stage of pregnancy, during the first trimester for asymptomatic women at risk of preterm birth, and for some asymptomatic women.

1. Introduction

Cervicovaginal infections have been associated with a series of pregnancy-related complications, including preterm delivery, premature rupture of the membranes (PROM), mid-trimester spontaneous abortion, intrauterine growth restriction, intrauterine death, neonatal infections, postpartum endometritis, and postoperative infections [1–6]. The most frequently observed microorganisms in the female genital tract are group B streptococcus, *Escherichia coli*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Chlamydia trachomatis*, and those responsible for bacterial vaginosis [3]. Increasing evidence suggests that PROM, preterm PROM, and neonatal sepsis are triggered by microorganisms such as *E. coli*, *C. trachomatis*, group B streptococcus, and abnormal vaginal flora (e.g. bacterial vaginosis) [5,7]. Recently, greater attention has been paid to aerobic vaginitis, which involves an abnormal vaginal microflora with an increased localized inflammatory reaction and immune response, unlike bacterial vaginosis [4].

The aim of the present study was to evaluate the prevalence of cervicovaginal infections and bacterial vaginosis in a large sample of pregnant women in Italy. Additionally, the relationship between abnormal vaginal flora and sociodemographic/clinical characteristics of women was assessed to identify pregnancies in which a cervicovaginal culture would be advisable.

2. Materials and methods

A retrospective study was undertaken using data from pregnant women who underwent a cervicovaginal smear test at Sant'Anna Hospital, Turin, between January 1, 2000, and December 31, 2010. Tests were performed at any pregnancy stage for patients who reported symptoms linked with genital infections and asymptomatic women at risk of preterm birth. Women with no genital symptoms or risk factors underwent cervicovaginal smears during the first obstetric visit. Given its retrospective nature, the study was exempted from ethics committee approval. Similarly, informed consent was not required.

Results of cervicovaginal smear microbiological testing and the sociodemographic/clinical characteristics of the women were obtained from the laboratory archives. Characteristics recorded included age, ethnic origin, education, marital status, sexual history (age at first intercourse, number of sexual partners, and sexually transmitted infections),

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obstetric history, and motivation for testing. The study sample was divided into three groups according to the presence of typical symptoms of lower-genital-tract infections: women in group 1 (G1) were symptomatic, with vaginal discharge and vulval or vaginal complaints; those in group 2 (G2) had no symptoms of lower-genital-tract infections but had risk factors for preterm birth, including uterine contractions and cervical ripening; and those in group 3 (G3) had no genital symptoms or risk factors.

Vaginal swabs were used in all cervicovaginal smears to evaluate the presence of bacterial vaginosis, *Candida* spp., *T. vaginalis*, and/or aerobic vaginitis, whereas endocervical samples were used for assessment of *C. trachomatis*, *U. urealyticum*, and/or *M. hominis*. Not all microorganisms were assessed in each patient; which tests were done was decided on the basis of the patient's history and physical examination. Vaginal smears, collected from the posterior fornix, were initially analyzed by electronic microscopy at 40 × magnification to identify *T. vaginalis* and *Candida spp*. If positive, a vaginal swab was placed in transport media and cultured by standard laboratory methods for *Candida* spp. and Gram-negative/positive bacteria, followed by Gram staining.

Candida spp. were isolated by selective culture on CHROMagar (Becton Dickinson, Franklin Lakes, NJ, USA) at 37°C for 48 hours and identified on the appearance of green colonies. A diagnosis of bacterial vaginosis was based on the presence of three of four of the Amsel criteria: a thin, white, yellow, homogeneous discharge; clue cells on microscopy; a vaginal fluid pH of above 4.5; and the release of a fishy odor on adding 10% potassium hydroxide solution [8]. Genital mycoplasma were diagnosed using Mycofast Evolution 3 broth culture (ELITech Group, Puteaux, France); the culture was considered positive at a more than 103-unit color change. *C. trachomatis* was identified by transcription mediated amplification with a DNA probe designed to detect ribosomal RNA gene amplicons of *C. trachomatis* in endocervical samples (Gen-Probe AMP CT TMA test for *Chlamydia*; Gen-Probe Incorporated, San Diego, CA, USA). The determination of aerobic vaginitis was established according to the criteria previously defined by Donders et al. [9]: the score is calculated with the use of high-power field microscopy to evaluate the presence or absence of healthy *Lactobacilli*, the number of leukocytes and toxic leukocytes, the type of vaginal flora, and the number of parabasal epithelial cells. A normal vaginal flora is characterized by the presence of sufficient *Lactobacilli* to maintain a normal vaginal pH of 4.5.

Tests showing the presence of multiple microorganisms were excluded from the analyses to reduce the bias. The data obtained were compared by either the χ^2 or Fisher exact tests. Univariate analyses were performed for each of the risk factor variables to ascertain the crude odds ratios (ORs) and the 95% confidence intervals (CIs), as estimated by logistic analysis. Adjusted ORs and 95% CIs were calculated by multiple logistic regression models to identify any variables independently associated with each microorganism. Cases with incomplete data were eliminated from the logistic regression models. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS version 20.0 (IBM, Armonk, NY, USA).

3. Results

During the study period, 11 219 pregnant women underwent a cervicovaginal smear test. Mean age was 31 ± 5 years. Overall, 4083 (36.4%) were symptomatic (G1), 1137 (10.1%) were asymptomatic but at risk of preterm birth (G2), and 5999 (53.5%) were asymptomatic and without risk (G3). Characteristics of included women are shown in Table 1.

A total of 4913 (43.8%) tests were positive for cervicovaginal infections and/or bacterial vaginosis. The frequency of positive tests was significantly higher in G1 (2145 [52.5%] women) and G2 (535 [47.1%]) than in G3 (2233 [37.2%]; $P < 0.001$ for both). The most common microorganisms were yeast (identified in 1669 [14.9%] of 11 209 samples tested), *U. urealyticum* (1076/10 668 [10.1%]), aerobic microflora (797/10 769 [7.4%]), *C. trachomatis* (37/10 544 [0.4%]), *M. hominis* (16/10 668 [0.1%]), and *T. vaginalis* (13/10 825 [0.1%]). Bacterial vaginosis was noted for 188 (1.7%) of 11 067 tests.

Table 1
Characteristics of symptomatic (group 1), asymptomatic at risk (group 2), and asymptomatic (group 3) pregnant women.[a,b]

Characteristics	Group 1 (n = 4083)	Group 2 (n = 1137)	Group 3 (n = 5999)
Age, y			
14–25	707 (17.3)[c]	172 (15.1)[d]	575 (9.6)
26–35	2691 (65.9)	773 (68.0)	4281 (71.4)
>35	685 (16.8)	192 (16.9)	1143 (19.1)
Country of origin			
Italy	3574 (90.1)	735 (86.4)	5009 (90.8)
Outside Italy	395 (10.0)	116 (13.6)[d]	505 (9.2)
Western Europe	86 (2.2)	21 (2.5)	122 (2.2)
Eastern Europe	82 (2.1)	23 (2.7)	112 (2.0)
South America	73 (1.8)	14 (1.7)	61 (1.1)
North Africa	85 (2.1)	30 (3.5)	103 (1.9)
Central West Africa	42 (1.1)	20 (2.4)	64 (1.2)
Asia	27 (0.7)	8 (0.9)	43 (0.8)
Education, y			
0–8	1513 (39.1)[c]	326 (42.8)[d]	1597 (29.6)
9–13	1822 (47.1)	333 (43.8)	2732 (50.7)
>13	531 (13.7)	102 (13.4)	1062 (19.7)
Marital status			
Married	3229 (82.7)	692 (87.0)	4711 (86.6)
Not married	675 (17.3)	103 (13.0)	730 (13.4)
Parity			
Primiparous	1851 (46.5)	403 (49.0)	2836 (51.2)
Previous spontaneous abortion(s) only	627 (15.8)	119 (14.5)	823 (14.9)
Multiparous without preterm birth	1442 (36.2)[c]	276 (33.6)	1805 (32.6)
Multiparous with preterm birth	62 (1.6)	24 (2.9)[c]	72 (1.3)
Past spontaneous abortions			
No	3311 (83.9)	667 (82.1)	4654 (84.4)
Yes	637 (16.1)	145 (17.9)[d]	859 (15.6)
Past induced abortions			
No	3380 (85.6)	723 (89.3)	4917 (89.1)
Yes	569 (14.4)[c]	87 (10.7)	600 (10.9)
Sexual history			
Age at first intercourse, y			
≤15	314 (9.6)[c]	58 (10.1)[d]	321 (6.8)
>15	2969 (90.4)	519 (90.0)	4403 (93.2)
No. of sexual partners			
1	1427 (44.3)	274 (48.4)	2057 (44.5)
2–5	1512 (46.9)	246 (43.5)	2189 (47.4)
≥6	284 (8.8)	46 (8.1)	375 (8.1)
Previous sexually transmitted infection			
Yes	653 (16.0)[c]	82 (7.2)	604 (10.1)
No	3430 (84.0)	1055 (92.8)	5395 (89.9)

[a] Values are given as number (percentage).
[b] The denominators used to calculate the percentages vary; some data were missing for some patients and variables.
[c] Comparison between group 1 and group 3: $P < 0.001$.
[d] Comparison between group 2 and group 3: $P < 0.001$.

Tests showing infection with multiple microorganisms (1130 [23.0%]) were excluded. One microorganism was observed in 3783 (77.0%) of the positive tests.

Fig. 1 shows the frequency of single microorganisms isolated in all three groups. The prevalence of *Candida spp.* was much higher in G1 (893/4080 [21.9%]) than in G2 (120/1135 [10.6%]; OR 2.4, 95% CI 1.94–2.91; $P < 0.001$) or G3 (656/5994 [10.9%]; OR 2.3, 95% CI 2.04–2.55; $P < 0.001$). The prevalence of bacterial vaginosis was higher in G1 (81/3982 [2.0%]) than G3 (83/5956 [1.4%]; OR 1.4, 95% CI 1.06–1.96; $P = 0.014$). Despite a high frequency of bacterial vaginosis in G2 (24/1129 [2.1%]), it did not differ significantly from G3 ($P = 0.086$). The prevalence of *U. urealyticum* was significantly higher in G2 (143/1106 [12.9%]) than in G3 (582/5828 [10.0%]; OR 1.3, 95% CI 1.10–1.63; $P = 0.0034$) or G1 (351/3734 [9.4%]; OR 1.4, 95% CI 1.16–1.76; $P < 0.001$). Finally, aerobic vaginitis was significantly more frequent in G2 (102/1123 [9.1%]) than in G3 (428/5847 [7.3%]; OR 1.4,