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Original article

A prospective observational study of vulvovagintis in pregnant women in Argentina, with special reference to candidiasis

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Summary

To evaluate the frequency of yeast, bacteria or protozoa in pregnant women and to correlate the possible associations of these microorganisms and their relationships with vulvovaginitis (VV) and cervicitis. Vaginal specimens were collected and prepared for smears in microscope slides for the evaluation of yeast, *Trichomonas vaginalis* and bacteria. Samples were cultured in specific culture medium. Cervical specimens were used to investigate the presence of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma* spp. and *Mycoplasma hominis.* We enrolled 210 pregnant women, aged 10–42 years old. Of them, 38.1% were symptomatic. Symptoms were most prevalent in the second and third trimesters of pregnancy coincident with a major prevalence of microorganisms. In this study, 39.5% of pregnant women had normal microbial biota and symptoms of VV due to non-infectious causes were observed (6.2%). The occurrence of vulvovaginal candidiasis was 25% and *Candida albicans* with a prevalence of 80.7% was the dominant species ($P = 0.005$) while non-*albicans Candida* species and other yeast were more common in asymptomatic ones ($P = 0.0038$). The frequency of bacterial vaginosis, *T. vaginalis, C. trachomatis* and *N. gonorrhoeae* were 18.1%, 1.4, 1.4% and 0.5% respectively.

Key words: Pregnant women, vulvovaginal candidiasis, bacterial vaginosis, *Trichomonas vaginalis*, Argentina.

Introduction

Acute vulvovaginitis (VV) is the most frequent infection of the female genital tract and is characterised by vaginal discharge, vulvovaginal itching, inflammation, burning sensation, dysuria and dyspareunia. VV can have a fungal, protozoal, viral or bacterial aetiology.[1] In this regard, bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and the infection with *Trichomonas vaginalis*, are among the major aetiologies of nearly 90% of the microbiological diagnosis of VV.[1]

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Bacterial vaginosis was recognised as a vaginal syndrome 60 years ago[2] and is produced by a disturbance in the vaginal microbiota, where lactobacilli are the dominant group of microorganisms in the 'normal flora' of the adult women in their reproductive years.[1,3] The microbiology of BV is complex and involves organisms other than *Gardnerella vaginalis*, which is often isolated from vaginas of 20–40% of women without BV. The anaerobic Gram-negative rods belonging to the genera *Prevotella*, *Porphyromonas*, *Bacteroides*, *Peptostreptococcus* species, *Mycoplasma hominis*, *Ureaplasma urealyticum* and often *Mobiluncus* species are usually present in high concentrations together with *G. vaginalis* in the vaginas of women with BV.[4–6]

Bacterial vaginosis is believed to be a factor in the development of sexually transmitted diseases (STDs), preterm birth, pelvic inflammatory disease and infertility.[7] *Chlamydia trachomatis*, *Ureaplasma* spp. and *M. hominis* are also associated with vaginitis. *T. vaginalis* infection is a common sexually transmitted

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protozoa infection. This protozoan is associated with several adverse health outcomes, including preterm birth, delivery of a low-birth weight infant and facilitation of sexual transmission of human immunodeficiency virus (HIV) and probably hepatitis B virus (HBV) since both viruses share the same transmission routes.[4,6]

Although most women with VV assume that *Candida* is the cause, this is only 15–30% of cases.[1] The asymptomatic carriage of *Candida* spp. in vagina of non-pregnant women is approximately 10–20% while in pregnant women, these numbers are higher up to 35%.[6] Most cases of VVC are caused by *C. albicans*.[1,6,8–10]

It is widely known that microorganisms, such as *C. albicans* and *G. vaginalis* which are considered as habitual constituents of the vaginal microbiota can become pathogenic when abnormal growth in the mucosa of the female genital tract is produced.[3]

This research was developed to evaluate the frequency of yeast, bacteria or protozoa in pregnant women and to correlate the possible associations of these microorganisms and their relationships with symptoms in VV and cervicitis.

Materials and methods

This study was carried out in the Laboratory of Microbiology of the 'Ramon Sarda' Maternity Hospital in Buenos Aires, Argentina, during the period of August 2012 to January 2013 and in *the* Mycology Center, School of Medicine, University of Buenos Aires. This research was conducted on those pregnant and immunocompetent women who visited the 'Ramon Sarda' Maternity Hospital for examinations of vaginal secretion, independent of the presence or absence of symptoms of VV. The vaginal discharge was characterised when sample was taken according to Odds *et al.* [11]. All women involved in the research signed a term of consent in which they declared to know that the collected material would be used for research, as mandatory by the Ethical Committee of 'Ramon Sarda' Maternity Hospital. All patients answered a standard questionnaire on their personal data such as age, trimester of gestation, clinical history of medical concern, antibiotic and antifungal therapy and symptoms of VV (vaginal discharge, vulvovaginal itching, vulvovaginal burning sensation, dysuria and dyspareunia).

Vaginal and cervical specimens were collected using sterile swabs with the aid of a disposable vaginal speculum. At this time, the appearance and type of vaginal secretions were recorded. The samples were prepared for smears in microscope slides, which were directly observed on saline wet mounts and dyed by Gram-Nicolle and Giemsa stains for the evaluation of the presence of yeast, *T. vaginalis* and bacteria.[6,12]

Specific yeast were initially phenotypically identified using conventional methods, such as the appearance of the colonies grown on the chromogenic medium CHROMagar *Candida*® (CHROMagar, Paris, France) at 37 °C for 48 h, and the micromorphological studies in milk agar plates supplemented with 1% between 80.[13] Those isolates that grew as light-green colonies were screened for chlamydospore formation on Staib agar as a species-specific characteristic of *C. dubliniensis*.[14,15] Individual isolates that grew as purple colonies with no pseudohyphae production[13] were evaluated for the presence of *C. glabrata* based on trehalose and sucrose assimilation using Rosco diagnostic tablets (Rosco Diagnostica A/S, Dk 2630 Taastrup, Denmark).[16] The isolates that grew as white colonies with no pseudohyphae production[13] were examined for the presence of *Saccharomyces cerevisiae* based on ascospore formation. Studies on ascospore formation by *S. cerevisiae* were carried out on potassium acetate agar medium during 10 days at 28 °C following by Kinyoun staining for the visualization of ascospores.

Finally, yeast were identified using a standard system, API ID32C (BioMérieux, Lyon, France).

To make the diagnosis of BV, a swab from inside the vagina was spread in Petri dishes containing the culture medium Columbia agar supplemented with 5% sheep blood (BioMérieux, France) during 48 h at 37 °C in a 5% CO_2 atmosphere. BV was diagnosed using Nugent criteria[17] and by detecting the presence of clue cells.[12]

Additional cervical specimens collected with Dacron swabs were used to investigate the presence of *N. gonorrhoeae*, *C. trachomatis*, *Ureaplasma* spp. and *M. hominis*. For these purposes, one of the samples was inoculated on Thayer Martin medium (BioMérieux) and was incubated for 48 h at 37 °C in a CO_2-enriched atmosphere. Another sample was also collected for direct immunofluorescence (IF) using monoclonal antibodies (Chlamydia direct IF, BioMérieux) for the detection of *C. trachomatis*. Finally, the commercial Mycoplasma-IST 2 kit (BioMérieux) was used for the detection of *Ureaplasma* spp and *M. hominis*. Isolated bacteria were then phenotypically characterised using conventional methods.[18–20]

Statistical methods

Statistical analysis of the data was performed using GRAPHAD PRISM version 6.00 for WINDOWS (GraphPad

Software Inc., La Jolla, California, USA). Statistical significance was set at $P < 0.05$.

Results

Demographic characteristics and risk factors

The median age of the pregnant patients with VV was 27.9 [SD 6.5] years old. Of the 210 studied patients, 16 (7.6%) were in the first trimester of pregnancy (weeks 1–12) while 126 (60%) were in the second trimester (weeks 13–28) and 68 (32.4%) in the third trimester of pregnancy (weeks 29–40). In total, six (2.8%) patients had a significant underlying disease (diabetes, Chagas disease, asthma, salpingitis, antiphospholipid syndrome and pregnancy-induced hypertension). In the last two weeks prior to the collection of the vaginal/cervical samples, nine (4.2%) and two (0.9%) patients had received antibiotic and glucocorticosteroid therapy, respectively, prior to the development of VV.

Complications of pregnancy

Overall, 82 (39%) of the pregnant women enrolled in this study had obstetrical complications. Among them, 47 patients (57.3%) suffered from threat of premature labour (TPL), 15 (18.3%) had vaginal discharge, 10 (13.4%) presented premature rupture of the amniotic membrane (PRAM), five (6.1%) suffered from spontaneous abortion (miscarriage), two had metrorrhagia (1%), one (0.5%) suffered foetal death and one (0.5%) presented chorioamnionitis. Furthermore, more symptomatic pregnant women had recurrent vaginal discharge and more asymptomatic pregnancy presented TPL (data not showed) ($P < 0.05$). There were no differences between symptomatic and asymptomatic women with respect to PRAM.

Clinical manifestations

Of a total of 210 pregnant patients enrolled in this study, 80 (38.1%) were symptomatic. Symptoms were more prevalent in the second and third trimesters of pregnancy. Vaginal discharge (100.0%, 80/80), vulvovaginal itching (46.2%, 37/80), vulvovaginal burning sensation (22.5%, 18/80) and dysuria (6.2%, 5/80) were among the most common described symptoms. None of the pregnant women analysed referred dyspareunia. Regarding to the appearance of the vaginal discharge of symptomatic patients, a clear vaginal discharge was frequent among asymptomatic patients ($P < 0.0001$) while white vaginal discharges were more frequent among symptomatic patients ($P < 0.0001$). Microscopically, polymorphonuclear inflammatory infiltrates were observed in 85.2% of the symptomatic patients.

In this study, 39.5% (83/210) of pregnant women had normal microbial biota (Tables 1 and 3). Symptoms of VV due to non-infectious causes were observed in 6.2% (13/210) of the patients.

Mycological data

Direct examinations of 32.9% (69 patients) of the vaginal/cervical swabs collected in this study revealed the presence of yeast or yeast with pseudohyphae. Conversely, CHROMagar *Candida* ® used in presumptive identification and inoculated with the swabs yielded yeast in 33.3% (70 patients) (Table 1). In both –microscopic and culture examinations – positive findings were mainly achieved in the symptomatic group ($P = 0.0001$). These patients suffered from VVC. Yeast recovered from CHROMagar Candida® cultures were then identified by morphological and biochemical features and through the use of the standard system, API ID 32C. Overall results demonstrated that 70 yeast species, mostly *Candida* were isolated from the 210 pregnant women. A total of three of these 70 (4.3%) positive cultures contained mixtures of species (Table 2).

Distribution of yeast from the vaginal/cervical discharges were as follows: *C. albicans* was the most frequent species isolated from 51 samples (72.8%), followed by *Rhodotorula* spp. from seven (10%), *C. dubliniensis* from three (4.3%), *C. glabrata* from

Table 1 Microbial diagnosis from vaginal/cervical samples of symptomatic ($n = 80$) and asymptomatic ($n = 130$) pregnant women ($n = 210$).

Microbial diagnosis	Symptomatic patients *n* (%)	Asymptomatic patients *n* (%)	Total *n* (%)
BV[1]	15 (18.7)	23 (17.6)	38 (18.1)
Candidiasis	52 (65.0)	18 (13.8)	70 (33.3)
Trichomoniasis	3 (3.7)	0	3 (1.4)
Gonorrhoea	0	1 (0.8)	1 (0.5)
C. trachomatis infection	1 (1.2)	2 (1.5)	3 (1.4)
M. hominis infection	1 (1.2)	5 (3.8)	6 (2.8)
Ureaplasma spp. Infection	15 (18.7)	22 (16.9)	37 (17.6)
Negative	13 (16.2)	70 (53.8)	83 (39.5)

[1]BV, bacterial vaginosis.

Table 2 Distribution of yeast (n = 70) between symptomatic (n = 52) and asymptomatic patients (n = 18).

Yeast species	Symptomatic patients *n* (%)	Asymptomatic patients *n* (%)	Total *n* (%)
C. albicans	42 (80.7)	9 (50.0)	51 (72.8)
Rhodotorula spp.	2 (3.8)	5 (27.8)	7 (10.0)
C. dubliniensis	2 (3.8)	1 (5.5)	3 (4.3)
C. glabrata	2 (3.8)	1 (5.5)	3 (4.3)
C. parapsilosis	1 (1.9)	0 (0.0)	1 (1.4)
C. incospicua	0 (0.0)	1 (5.5)	1 (1.4)
S. cerevisiae	0 (0.0)	1 (5.5)	1 (1.4)
C. albicans/ *C. glabrata*	2 (3.8)	0 (0.0)	2 (2.8)
C. albicans/ *C. tropicalis*	1 (1.9)	0 (0.0)	1 (1.4)
Total	52 (100)	18 (100)	70 (100)

three (4.3%), *C. parapsilosis* from one (1.4%), *C. incospicua* from one (1.4%), *S. cerevisiae* from one (1.4%), and mixtures of yeast species such as the associations *C. albicans*/*C. glabrata* and *C. albicans*/*C. tropicalis* were observed in two (2.8%) and in one (1.4%) clinical specimens respectively. Table 2 shows the distribution of yeast species between symptomatic (patients with VVC) and asymptomatic patients (carrying vulvovaginal yeast). Thus, *C. albicans* was more frequent among the symptomatic pregnant women (P = 0.005) while *non-albicans* species and others yeast were more frequent in asymptomatic ones (P = 0.0038).

Yeast isolation was higher in the second and third trimester of pregnancy than in the first one (85.7% vs. 14.3%).

Bacterial data

Bacterial vaginosis was detected in 38 out of 210 patients analysed (18.1%) (Table 1). Among them, 39.5% (15/38) were symptomatic. The 100% of the cases of BV was observed in the second and third trimester of pregnancy. Co-infections with *Candida* spp. occurred in eight patients (21.0%); seven with *C. albicans* and the remaining one with *C. glabrata* (Table 3).

In six out of 210 pregnant patients (2.8%), *M. hominis* was identified. Among them, one patient (16.7%) was symptomatic. The recognition of *M. hominis* was higher in the second and third trimester of pregnancy than in the first one (83.4% vs. 16.6%). In this case, co-infection with *C. albicans* and *Ureaplasma* spp. was found (Tables 1 and 3).

In 37 out of 210 pregnant patients (17.6%), *Ureaplasma* spp. was observed from cervical samples (Table 1). Symptoms were present in 40.5% of women (15/37). The identification of *Ureaplasma* spp (91.9%) was done in the second and third trimester of pregnancy. Co-infections of *Ureaplasma* spp with *Candida* spp. occurred in 29.7% (11 patients, seven with *C. albicans*, two with *C. dubliniensis* and two with *C. glabrata*). In addition, co-infections of *C. albicans* with BV and *C. albicans* with *M. hominis*, also occurred both in 2.7% (Tables 1 and 3).

In three out of 210 pregnant women (1.4%), *C. trachomatis* was detected from cervical samples (Table 1). Symptoms were present in only one patient (33.3%) that referred as bothering the vaginal discharge. A 100% of these three patients had a clear vaginal discharge. No co-infections were observed (Tables 1 and 3).

Table 3 Microorganisms isolated from vaginal/cervical samples of symptomatic (n = 80) and asymptomatic (n = 130) pregnant women.

Microorganisms	Symptomatic patients *n* (%)	Asymptomatic patients *n* (%)	Total *n* (%)
Candida spp.	36 (45.0)	15 (11.5)	51 (24.39)
Candida spp. + *Ureaplasma* spp. + *M. hominis*	1 (1.2)	0	1 (0.5)
Candida spp. + *Ureaplasma* spp.	8 (10.0)	1 (0.8)	9 (4.3)
Candida spp. + BV[1]	6 (7.5)	2 (1.5)	8 (3.8)
Candida spp. + BV + *Ureaplasma* spp.	1 (1.2)	0	1 (0.5)
BV[1]	7 (8.8)	16 (12.3)	23 (11.0)
BV[1] + *M. hominis*	0	2 (1.5)	2 (1.0)
BV[1] + *Ureaplasma* spp.	1 (1.2)	3 (2.3)	4 (1.9)
T. vaginalis	2 (2.5)	0	2 (1.0)
T. vaginalis +*Ureaplasma* spp.	1 (1.2)	0	1 (0.5)
Ureaplasma spp.	3 (3.8)	16 (2.3)	19 (9.0)
Ureaplasma spp. + *M. hominis*	0	2 (1.5)	2 (1.0)
M. hominis + *N. gonorrhoeae*	0	1 (0.8)	1 (0.5)
C. trachomatis	1 (1.2)	2 (1.5)	3 (1.4)
None	13 (16.2)	70 (53.8)	83 (39.5)
Total	80 (100.0)	130 (100.0)	210 (100.0)

[1]BV, bacterial vaginosis.

Protozoal data

Trichomonas vaginalis was documented in three out of 210 pregnant women (1.4%) enrolled in this study. All of them had symptoms. No co-infections were observed (Tables 1 and 3).

Discussion

The vaginal environment is particularly sensitive to oestrogen, a hormone that induces considerable changes in the vaginal microbiota. In pregnant women, levels of oestrogen rise steadily over the course of pregnancy.[3,5] This hormone is known to stimulate the intracellular glycogen deposition, which is metabolised to glucose, the main carbon source for fungal and bacterial growth.

Disturbances in the vaginal microbiota can be a prelude to the development of BV, VVC and other diseases.[3] Furthermore, the introduction of a new pathogen does not always result in disease while microorganisms that are recognised as part of the vaginal microbiota (i.e. *C. albicans*, *G. vaginalis*), can become pathogenic when vaginal homeostasis is disturbed.[5,21]

Non-infectious vaginitis was observed in the pregnancy population studied and it is often caused by an allergic reaction or irritation from a manufactured product or activity, such as douching, vaginal sprays, spermicidal products, soaps, detergents or fabric softeners.[6,22,23]

In 100% of symptomatic women, vaginal discharge was documented[11] and symptoms were prominent over the course of pregnancy, being more pronounced in the second and third trimester of gestation, possibly as a consequence of the higher colonisation of the genital tract by *Candida* spp, *M. hominis*, *Ureaplasma* and VB, and the disturbed homeostasis leading to vaginitis or cervicitis.[5]

Reliable diagnosis of VVC requires a correlation of clinical features with mycological evidence.[21] Thus, in this study, the prevalence of VVC in pregnant women was of 25% (52/210). Similar results were obtained by Garcia-Heredia *et al.* [8] (28%), although higher percentages were reported.[10,24,25] Many recent studies have demonstrated that the prevalence of *C. albicans* is from 40.5% to 85.0%.[1,26] These differences may reflect the population analysed in such studies (i.e. refractory VVC patients, infertile women, pregnant women).

In this report, *C. albicans* was the predominant *Candida* species accounting for 80.7% (42/52) of VVC cases. Non-*C. albicans* yeast were isolated in 19.3% cases of VVC. The overall percentage of non-*C. albicans* species or other yeast associated with VVC ranged from 11% to 24%.[26]

The presence of yeast in vagina without signs of disease is referred to as colonisation.[26] In patients who are asymptomatic carriers of yeast, the results of microscopic examinations of vaginal secretions tend to be negative; colonisation is usually identified on the basis of a positive culture for yeast.[21]

The isolation of non-*C. albicans* species and other yeast (*Rhodotorula* spp. *S. cerevisiae*) were higher in asymptomatic women (50%) than in patients suffering from VVC. These findings suggest that in the vagina, colonisation by yeast is associated with non-*C. albicans* yeast and that the evolution to the symptoms of VVC would depend, among other factors, on replacement by *C. albicans.*[27] In addition, *C. albicans* was a commensal agent in asymptomatic women (50%). In this case, development of VVC could depend on changes in the vaginal environment.[21]

In VVC, the most commonly detected non-*C. albicans* species were *C. glabrata* (3.8%), *C. dubliniensis* (3.8%), and *C. parapsilosis* (1.9%) and the association *C. albicans*/*C. glabrata* (3.8%) and *C. tropicalis*/*C. parapsilosis* (1.9%). Regarding *C. glabrata*, in this study, the depicted percentage was lower than the reported by other authors (3.8% vs. 6–16.5%).[8,9,21,23,28] *C. dubliniensis* shares many phenotypic similarities with *C. albicans.* These parallels carriage problems in the identification of *C. dubliniensis* and have previously led to misidentification.[14,15] Thus, prevalence values reported in VVC by *C. dubliniensis* are scarce and it is from 0.17 to 2.44%.[29,30]

As it was previously reported,[26] a single species was identified in most vaginal specimens, but two or more species have been found in the same vaginal culture in a minority of women (2–5%). In fact, VVC is rarely caused by *C. parapsilosis*, *C. tropicalis* and *C. krusei.*[21]

Rhodotorula was most frequently isolated from the asymptomatic group of pregnant women. In the last few years, *Rhodotorula* spp. have emerged as opportunistic pathogens that have the ability of colonise and infect susceptible patients.[31] Taking into account that *Rhodotorula* is a ubiquitous and saprophytic fungus, its isolation from no sterile human sites, especially from the mucous membranes, has often been of questionable clinical significance.[31] Nevertheless, it was reported as a cause of hydrosalpinx due to chronic VV.[32] In this study, *Rhodotorula* spp was obtained by culture and the yeast and polymorphonuclear leucocytes infiltration were observed on direct examination of vaginal discharge of symptomatic patients. Therefore, the finding in symptomatic patients is difficult to interpret because *Lactobacillus* in Gram Nicolle stain was observed and these microorganisms are believed to promote a healthy ecosystem.[1,5,21] *S. cerevisiae* is also a leading cause of VVC[23], but it was only

recovered from asymptomatic patients in a low percentage (1.4%).

Not infrequently, mixed infections occurred in which two simultaneous pathologic processes (e.g. bacterial plus candidal) are observed. Thus, in this study co-infections were documented, including *Candida* spp. with BV, *Ureaplasma* spp. and *M. hominis*.[8,23]

In the present report, the prevalence of trichomoniasis was slow (1.4%) as it was indicated previously and all of the patients with this protozoan were symptomatic.[1] It is known that *T. vaginalis* causes acute vaginitis in 5–50% of cases, depending on the population studied.[1] It has been observed that the prevalence of trichomoniasis is diminishing.[23] The low incidence documented in this study was probably due to the better hygiene practices implemented and the use of mechanical barriers to prevent sexually transmitted infections.

The prevalence of BV in this study was 18.1%; of them, 39.5% were symptomatic. According to the international literature, the prevalence of BV is between 6.4% and 38.0%, and it is often documented in asymptomatic women.[1,4,24,33]

The occurrence of STDs due to *C. trachomatis* and *N. gonorrhoeae* was low in the group of pregnant women analysed in this study. In this regard, and in accordance with other reports, the frequency of *C. trachomatis* in pregnant women was of 1.4%.[24] Consistent with the international literature, most cases of chlamydia infection were asymptomatic and no cases of co-infections with *Candida* spp. were observed.[24]

The prevalence of *N. gonorrhoeae* was 0.5%, similarly to reported data in our medium.[24]

In addition, low value of gonorrhoea was observed in HIV-positive women [23] probably due to the use of mechanical barriers to prevent sexually transmitted infections.

Finally, the prevalence of *Ureaplasma* spp. and *M. hominis* in the present work was of 17.6% and 2.8% respectively. Those pathogens are part of the vaginal microbiota and an increase in the number of these microorganisms during pregnancy is associated with obstetrical and perinatal complications. Pregnant women are at high risk of vaginal infections especially in the second and third trimesters. Adequate investigation and prompt treatment instauration will prevent adverse effects on mother and foetus (an increased risk of infection with HIV, preterm labour, low weight at birth, puerperal endometritis, conjunctivitis, pneumonia and sepsis secondary to infection in neonate, among others).[1,4,6,7]

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