Original Article

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Prevalence of *Trichomonas vaginalis* and *Candida albicans* among Brazilian Women of Reproductive Age

Community Medicine Section

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ABSTRACT

Introduction: There are no studies assessing the simultaneous occurrence of *Candida albicans (C. albicans)* and *Trichomonas vaginalis* (*T. vaginalis)* in the primary health care in Brazil. Despite different conditions to establishment of each one, the co-detection of both has been reported by some authors in previous studies from other regions.

Aim: To compare the prevalence of *T. vaginalis* and *C. albicans* in correlation with associated variables.

Materials and Methods: A cross-sectional study conducted in a family health clinic in the Federal District of Brazil, between November 2014 and March 2015. Vaginal swabs were collected from 201 women of the reproductive age selected from women registered at the family health clinic. Minors and pregnant women were excluded. The rates of *T. vaginalis* and *Candida albicans* prevalence were evaluated with vaginal pH, the whiff test, sexual practices and other social and demographic variables. Difference between proportions was assessed by Z-Test.

Results: *C. albicans* was present in 20% of the women, while 16% of them had *T. vaginalis*. The simultaneous occurrence of the agents was found in 1.5%. Significant differences were found between prevalence rates for the variables race/skin colour, practice of anilingus and lifetime number of sexual partners.

Conclusion: The prevalence of *T. vaginalis* exceeds *C.albicans* among women with higher numbers of sexual partners. The prevalence of *C. albicans* was higher than *T. vaginalis* among white women and those who practice active and receptive anilingus. The simultaneous occurrence of the two microorganisms was uncommon.

Keywords: Reproductive tract infections, Sexually transmitted infections, Whiff test

INTRODUCTION

Vaginal infections are common among women of reproductive age [1]. They can be caused by sexually transmitted microorganisms or by some disorder resulting in the proliferation of endogenous microbiota [1]. Among the more frequent microorganisms are the protozoan *Trichomonas vaginalis* (T. *vaginalis)* [2] and *Candida albicans* (*C. albicans)* [3].

T.vaginalis is a flagellate protozoan, facultative anaerobic, extracellular human parasite transmitted by sexual intercourse [4,5]. Classic symptoms of trichomoniasis include greenish yellow malodorous vaginal discharge, accompanied (or not) by local irritation [2]. The vaginal pH of women with trichomoniasis is often above 4.5 [1,6]. However, more than half of those infected are asymptomatic [7]. Risk factors for infection by *T.vaginalis* are, in general, the same as for other sexually transmitted infections (STI): higher numbers of lifetime sexual partners [7] and irregular use of condoms [8,9]. In a study conducted in a primary care setting, the authors report a *T.vaginalis* prevalence of 2.6% [10], quite different of that estimated by the Brazilian Ministry of Health (14%), in a study carried out in six Brazilian capitals [11].

Fungi of the genus *Candida* are commensals, usually found colonizing on human skin and in the gastrointestinal and genitourinary tracts [12]. Among the causes of vulvovaginitis, *Candida* spp. infection is the second most prevalent, after bacterial vaginosis [1,13]. It is estimated that one in five women harbour *Candida* species and other varieties of fungi in the vagina [2] and three out of four women will present at least one episode of vulvovaginal candidiasis during their lifetimes [14,15].

Vulvovaginal Candidiasis (VVC) is defined as the presence of signs of genital irritation/inflammation combined with the presence of *Candida* spp. in the absence of other aetiologies [12]. Thus, the simple presence of this fungus is not a defined infection. The symptoms of VVC usually include pruritus, burning and curd like vaginal discharge [16]. *Candida* thrives better at pH values between 3.9 and 5.0 [17]. In general, in the case of VVC, vaginal pH is less than 4.5 [1].

The ability to alternate between different forms of yeast and pseudohyphae is determinant for *Candida* spp. pathogenesis [18,19]. This occurs in many situations including diabetes mellitus [20], pregnancy [21], vaginal douching [22] and the use of antibiotics [23]. Gunther et al., reported a prevalence of 18.8% of *Candida* spp. among diabetic women [20].

It is also possible that *C.albicans* infection, as well as other reproductive tract infections, can be influenced by sexual practices [24,25]. Considering that *C.albicans* is part of the intestinal microbiota, its permissible to hypothesize that women colonized in the anal region had more likelihood of having VVC and oral sex has been considered by some authors as a risk factor for developing vulvovaginitis by *Candida* [23].

Although conditions for the establishment of *T.vaginalis* in the genital tract differ from those required by *Candida* spp., the simultaneous presence of these two microorganisms has been found in some women [26,27]. As per our knowledge, in Brazil there is no report of this occurrence in the Primary Health Care (PHC).

To verify the co-existence of these two agents, this study compares the prevalence of *T.vaginalis* and *C.albicans* in correlation with vaginal pH, whiff test results, sexual practices and other social and demographic variables.

MATERIALS AND METHODS

Between November 2014, and March 2015, a cross-sectional study was conducted in the Estrutural City Primary Health Care Unit, located in one of the administrative regions of the Federal

District, Midwest region of Brazil. Vaginal swabs were collected for the investigation of *C.albicans* and *T.vaginalis*.

The study was approved by the Research Ethics Committee of the Health Department of the Federal District (CAAE 28186514.5.0000.5553).

A total of 201 women agreed to participate in the study. The sample was composed of women of reproductive age, except pregnant women and minors (less than 18 years), who were outpatients of the clinic. Women between 18 and 49 years of age were consecutively invited to participate, regardless of the reason for coming to the clinic.

The sample size was calculated considering the number of registered women in the clinic as a finite population, and assuming a 10% prevalence of *T. vaginalis* infection with a 5% confidence interval. An adequate sample was calculated to be composed of 116 participants. Predicting a loss of 20%, the sample was increased to 139 women. In carrying out the collection of data, a total of 201 women participated and 193 of them were examined. Eight of them were not examined for diverse reasons, like presence of menstrual flow or even desistence.

DATA COLLECTION

A structured questionnaire was applied by a trained female interviewer in which the participants were asked about complaints that had occurred during the past four weeks. The participants also were asked about sociodemographic information (age, education, smoking) and sexual practices. Following interrogation, all the participants were referred to the examination room, regardless of whether or not they have complaints.

During the physical examination, after introduction of the vaginal speculum, an indicator strip was used to assess the pH of the secretion. Graduated strips were used at intervals of 0.3 units, with pH ranging between 3.6 and 6.1 (pH-Fix®, Macherey-Nagel, Ref 92130). The strips were placed in the middle third of the right side wall of the vagina. After the change in colour they were compared with the standardized colour chart provided by the manufacturer.

The whiff test was performed by adding a drop of 10% KOH to the vaginal secretion, previously collected with an Ayre spatula and disposed on a glass slide. The test was considered positive with the release of a characteristic fishy odour. In sequence, a sterile swab was applied against the posterior side wall of the vagina and placed in TYM (Trypticase - Yeast extract - maltose) culture medium, as proposed by Diamond [28]. On the contra lateral wall of the vagina, another sterile swab was rubbed and subsequently immersed in Stuart transport medium.

Samples were transported to the Laboratory of Parasitology and Vector Biology of the Faculty of Medicine at the University of Brasilia. The plates with TYM culture medium were placed in an incubator at 37ºC. The swabs in the Stuart culture medium were plated in Sabouraud agar for isolation of fungi of the genus *Candida* spp.

The plates with TYM culture medium were examined after 24, 48 and 72 hours. After 96 hours, the culture was washed with 5 mL of 0.9% saline solution, transferred to test tubes and centrifuged at 2,500 g for five minutes. The supernatants were discarded and the remaining pellets were examined. For each sample, 100 µL of the pellets were placed on glass slides and examined under an optical microscope at 40X. A second slide was made and allowed to dry at room temperature. This second slide was fixed with methanol, stained with Giemsa and examined under the microscope at 100X. The results were considered positive when the presence of the parasite on either of the slides was verified [10,29].

The swabs immersed in Stuart medium were rubbed on plates with Sabouraud agar for the isolation of fungi of the genus *Candida*, as suggested by the manufacturer (Neogen, Michigan, USA). These plates were placed in an incubator (B.O.B, MA 415) at 28°C for 72 hours. After this time, the plates were examined under a loupe (Olympus SZ 40) and the Colony Forming Units (CFU) were counted. The samples were positive if they formed colonies of *Candida* spp as described in the literature [30].

Germ Tube Test

For determination of the *Candida* spp. species in the positive cultures, samples of the colonies were subjected to the germ tube test. This structure is a filament that sprouts from yeast, and whose presence allows the presumptive identification of *C.albicans* [30].

For this test, part of the colony was taken from the plate and placed in an Eppendorf microtube containing 5mL bovine fetal serum. After 30 minutes and within two hours, a drop of serum-yeast suspension was placed on the slides and they were observed under an optical microscope.

STATISTICAL ANALYSIS

Information was collected regarding age, years of education, race/skin colour, hormonal contraception, smoking, sexual practices, pH and whiff test results.

The race/skin colour variable was measured according to the self-declaration of each participant. After observing that more than half of the women declared that they were of mixed race or black, the variable was grouped into two categories (white and non-white).

The differences between the proportions were verified with a one tailed test, which was considered significant when Z-calculated > Z-critical. In the case of a one tailed curve, with an error of 5%, Z-critical equals 1.64.

RESULTS

The average age of the participants was 34 years (95% CI: 30.7 – 37.3), most of whom were of mixed races (58%). More than half of the women (54%) had eight years or less of formal education. Thirty-four women (17%) reported having had some Sexually Transmitted Disease (STD) at some point in life.

Gynaecological complaints were common among participants, predominantly lower abdominal pain (50%), and followed by reports of vaginal discharge (46%). Thirty women (30/193) were positive for *Trichomonas vaginalis* (16%; 95% CI: 11-21%) and among the women with positive samples, the most frequent complaint was vaginal discharge (57%). Of women with positive cultures, 27 (90%) had at least one complaint, although three (10%) of them had no complaints [Table/Fig-1]. The average number of lifetime sexual partners of the women with *T. vaginalis* was 9.4.

Thirty-nine women (39/193) were positive for *Candida albicans* and vaginal discharge. Itching and lower abdominal pain occurred in the same proportions (46%). Among women with positive cultures, six (15%) were completely asymptomatic [Table/Fig-1]. The average number of sexual partners of the women positive for *C. albicans* was 5.6.

The co-existence of *T.vaginalis* and *C.albicans* was observed in three women, representing 1.55% of the women examined. The rates of prevalence were similar in correlations between many

Complaints	n*	Tv Prevalence (%)	*Candida* Prevalence (%)	Variation	Zcalc
Vaginal discharge	92	0.18	0.20	0.01	0.11
Itching	56	0.18	0.32	0.14	1.40
Burning	54	0,20	0.26	0.06	0.55
Malodorours discharge	66	0.18	0.21	0.03	0.32
Dyspareunia	68	0.22	0.21	-0.01	-0.15
Lower abdominal pain	101	0.15	0.18	0.03	0.33
Previous STD	33	0.24	0.18	-0.06	-0.61

[Table/Fig-1]: Distribution of women, according to the rates of prevalence of *T. vaginalis, C. albicans* and gynaecological complaints.
*n: number of participants

of the variables, except three of them: 1) More than 10 partners throughout life – the prevalence of *T. vaginalis* (50%) exceeded the prevalence of *Candida* (19%) significantly; 2) The prevalence of *Candida* among women who practiced active (14%) and receptive (21%) anilingus was higher than the prevalence of *Trichomonas vaginalis* (0%); and 3) The prevalence of *C. albicans* exceeded the prevalence of *T. vaginalis* among white women – 26% versus 11%.

For the other variables, there were no significant differences between the prevalence of *C.albicans* and *T.vaginalis* [Table/Fig-2].

Characterisctics	n*	Tv Prevalence (%)	*Candida* Prevalence (%)	Variation	Z_{calc}
Sociodemographic					
Education up 8 years	102	0.19	0.17	-0.02	-0.21
Age > 30 years	120	0.17	0.30	0.13	1.33
White	**47**	**0.11**	**0.26**	**0.15**	**1.66†**
Non-white	146	0.17	0.18	0.01	0.15
Smoking	27	0.19	0.11	-0.07	-0.85
Oral contraceptive	39	0.13	0.13	0.00	0.00
Sexual intercourse < 15 years	63	0.24	0.11	-0.13	-1.37
Three or less partners	105	0.10	0.17	0.07	0.81
More than 10 partners	**16**	**0.50**	**0.19**	**-0.31**	**-2.82†**
Practices					
Anal intercourse	34	0.18	0.24	0.06	0.60
Oral active	86	0.15	0.20	0.05	0.51
Oral receptive	96	0.16	0.22	0.06	0.67
Anilingus (active)	**7**	**0.00**	**0.14**	**0.14**	**2.55†**
Anilingus (receptive)	**14**	**0.00**	**0.21**	**0.21**	**3.26†**
Duche	83	0.14	0.20	0.06	0.66
Condom use	35	0.20	0.17	-0.03	-0.30
Tests					
pH > 4,5	83	0.18	0.16	-0.02	-0.26
Whiff test	59	0.20	0.14	-0.07	-0.74

[Table/Fig-2]: Distribution of women, according to the rates of prevalence of *T. vaginalis* and *C.albicans* and sociodemographic variables, sexual practices, pH and the whiff test.
*n: number of participants; † significative differences.

DISCUSSION

In the present research, the rates of prevalence of *C.albicans* and *T.vaginalis* were not significantly different among women who practiced active or receptive oral sex. Oral sex has been appointed by some authors as a risk factor to develop VVC, but in this study there was no significant association among those who practiced oral sex and positivity for *Candida*.

In terms of the practice of oroanal sex, the prevalence of *Candida* was greater than the prevalence of *T. vaginalis* among women who practiced active and receptive anilingus. In fact, among women positive for *T. vaginalis*, there was no anilingus practice reported. However, the fact that such practice was uncommon, limited the statistical power to identify significant differences. The prevalence of *C.albicans* was greater than the prevalence of *T. vaginalis* among white women.

T. vaginalis is transmitted by sexual contact and its habitat is the human genital and urinary tract [1]. Due to being a classic Sexually Transmitted Disease (STD), one would assume that women with risk factors for STDs would have a higher prevalence of *T. vaginalis*. Higher numbers of lifetime sexual partners, for example, is a known risk factor for STDs [7]. In this study it was thus not a surprise that among women who reported more than 10 lifetime sexual partners, the prevalence of *T. vaginalis* significantly exceeded the prevalence of *C. albicans*.

Among the women of the sample, three were simultaneously positive for *C. albicans* and *T. vaginalis*. This represented 1.55% of the women examined.

The simultaneous encounter of both microorganisms have been reported by other authors [Table/Fig-3] [26,27,31-37], and varies from 0% [35] to 21.73% [31]. However, most of the studies were conducted in the secondary care and one included only symptomatic women [32]. One study conducted in the Primary Health Care (PHC) report 0,23% of mixed infection by *T. vaginalis* and *C. albicans* [26] while another one, also conducted in the PHC, reported a co-infection of 14% [27]. Both of these studies used molecular biology and the disparities can be in part explained by different populations studied. Even so, there is a lack of knowledge about the required conditions to the co-existence of these two microorganisms, given that they require different environmental needs.

Study replication with larger samples in different settings may help to better understand the distribution of these two microorganisms and their association with infrequent variables.

LIMITATION

A limitation of the study was the fact that sexual practices are part of the private life of the individual. Not all of the women in the study felt comfortable answering in a sincere manner, even when guaranteed confidentiality.

Authors	References	Year	Number of women	Age group (years)	Mean age (years)	*T. vaginalis* and *C. albicans* co-detection	
						n	%
Levi AW, Harigopal M, Hui P, Schofield K, Chhieng DC.	[26]	2011	431	17-79	33	1	0,23
Alo M, Anyim C, Onyebuchi A, Okonkwo E	[31]	2012	1500	20-40	not mentioned	326	21,73
López-Monteon A, Gómez-Figueroa FS, Ramos-Poceros G, Guzmán-Gómez D, Ramos-Ligonio A	[27]	2013	252	14-90	not mentioned	36	14,29
Arora BB, Maheshwari M, Devgan N, Arora DR (rural population)	[32]	2014	4672	15-76	not mentioned	294	6,3
Arora BB, Maheshwari M, Devgan N, Arora DR (urban population)	[32]	2014	7950	18-70	not mentioned	0	0
Ghaffari S, Bayani M, Kalantari N	[33]	2014	33600	17-73	34,7	46	0,14
Kadir MA, Sulyman MA, Dawood IS, Shams-Eldin S	[34]	2014	250	15-60	not mentioned	4	1,60
Olowe OA, Makanjuola OB, Olowe R, Adekanle DA	[35]	2014	100	21-39	28,1	0	0
Olorode OA, Mark OO, Ezenobi NO	[36]	2014	220	18-55	not mentioned	7	3,18
Nsagha DS, Zofou D, Assob JCN, Njunda AL, Nchang CD, MvoNgum N, et al	[37]	2015	249	15-56	28,7	3	1,20

[Table/Fig-3]: Studies reporting the co-detection of *C. albicans* and *T. vaginalis* in women [26,27,31-37].

CONCLUSION

We can conclude that the rates of prevalence of *T.vaginalis* and *C.albicans* in the women examined were high; however, the occurrence of co-infection with the two etiologic agents was not common. Among women with higher numbers of sexual partners during the lifetime, the prevalence of *T.vaginalis* exceeded the prevalence of *C.albicans*. The prevalence of *C.albicans* was higher than the prevalence of *T. vaginalis* among white women and among those who practiced active and receptive oral anal sex.

The possibility of using self-administered questionnaires may be an interesting alternative to increase the possibility of more accurate answers, if we take into account that some participants could feel uncomfortable to respond questions about their sexual practices.

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