

A Descriptive Study on the Prevalence of Vulvovaginal Infections and Species-specific Distribution of Vulvovaginal Candidiasis in Married Women of the South of Iran

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ARTICLE INFO	ABSTRACT
<p>Article type: Original article</p>	<p>Background & aim: As literature shows about 20% of non-pregnant women aged 15-55 harbour <i>Candida albicans</i> in the vagina. This study aimed to determine the characterization of <i>Candida</i> species isolated from women with vulvovaginal candidiasis during reproductive ages.</p>
<p>Article History: Received: 23- Sept -2015 Accepted: 7- Sept -2016</p>	<p>Methods: This descriptive study was conducted on 280 females who were selected through purposive sampling based on their history and characteristics of vaginal discharges in 2009. Among these patients, 105 females were diagnosed with vulvovaginal candidiasis. The data were collected using a form inquiring demographic characteristics, infection history, and infection features. The species were differentiated using germ tube test, CHROMagar test, and chlamyospore test. <i>Data</i> analysis was performed through descriptive statistics using SPSS 16.</p>
<p>Key words: <i>Candida</i> <i>C. albicans</i> Vaginitis Vulvovaginitis</p>	<p>Results: According the results of the study, the prevalence of <i>Candida</i> vaginitis was found to be 9.3%. Chlamyospore was detected on 54.3% of the corn meal agar media. Besides, in CHROMagar test, 41.9% of the samples turned into green representing <i>C. albicans</i>. On the other hand, in germ tube test, 70.5% of the samples were <i>C. albicans</i>, while 29.5% of them were <i>C. non-albicans</i>. Overall, the frequency of <i>C. albicans</i>, <i>C. glabrata</i>, <i>C. tropicalis</i>, and <i>C. Krusei</i> were 66.6%, 21.9%, 8.6%, and 2.9%, respectively.</p> <p>Conclusion: <i>C. albicans</i> was the most common species leading to the Vulvovaginitis in patients with VCC while other species were at the secondary importance stages. Due to inaccurate diagnosis of the disease based on the clinical symptoms, fungal culture is recommended as a standard diagnostic method.</p>

► Please cite this paper as:

Bonyadpour B, Akbarzadeh M, Mohagheghzadeh A. A Descriptive Study on the Prevalence of Vulvovaginal Infections and Species-specific Distribution of Vulvovaginal Candidiasis in Married Women of the South of Iran. 2016; 4 (4): 741-747. DOI: [10.22038/jmrh.2016.7562](https://doi.org/10.22038/jmrh.2016.7562)

Introduction

Vaginitis is the most prevalent disease among females, which is responsible for 10 million clinical visits every year (1-3). This disease is the second common genital tract infection among females and is usually caused by *C. albicans*. Nonetheless, the prevalence and causes of vaginitis are not known yet. Vaginitis is normally asymptomatic and has more than one etiology. Most specialists believe that 90% of cases of vaginitis are secondary to bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis (4). In addition, vaginitis may occur as the result of non-infectious causes,

such as vaginal atrophy, allergies, and chemical irritation (5).

Sometimes, vaginitis is accompanied by pelvic inflammatory disease, post-operative infections, abnormal cervical cytology, chronic cervicitis, and urinary tract infection (6). In England, Fai-Katty (1993) and Gardner (1998) reported the prevalence of *Candida* vaginitis to be 25% and 27%, respectively (7, 8). Besides, in a study conducted in Georgia University, Freeze et al. (1995) demonstrated that out of 499 cases with vaginitis 20%, 7.4%, 25%, and 14% were

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diagnosed with candidiasis, trichomoniasis, bacterial vaginosis, and combined infection, respectively (9).

Similarly, Bomsil et al. (2004) conducted a study on vaginal wet mount of 749 females. According to the microbiology results, 50.6%, 25.6%, 17.4%, 5.3%, and 0.3% of the cases had cytolytic vaginosis, *Gardnerella vaginalis*, *Candida* vaginitis, trichomoniasis vaginosis, and gonorrhoea, respectively (6).

C. albicans is the major cause of *Candida* infection in patients with vulvovaginitis, yet there are other species that are responsible for this kind of disease (10-13). Given the high prevalence of vaginitis, using serological techniques such as double diffusion method for diagnosis is helpful (14). Pirotta and colleagues' (2006) study showed that *C. albicans* was responsible for 75% of the infections, whereas other *Candida* species accounted for 25% of the cases (15).

A retrospective survey of 93,775 samples testing positive in *Candida* species-specific PCR tests demonstrated consistent yearly distributions of *Candida albicans* (89%), *C. glabrata* (7.9%), *C. parapsilosis* (1.7%), and *C. tropicalis* (1.4%) (5). Likewise, in some other studies, vulvovaginal candidiasis was demonstrated to have the highest frequency, compared to other vaginitis. (16-20).

According to the literature, the prevalence of recurrent *Candida* vaginitis and treatment-resistant cases have increased. Since most of these cases are induced by *C. non-albicans* species (15), it is essential to determine the prevalence rate of the infection and accurately identify *Candida* species responsible for *Candida* vaginitis. The inappropriate use of anti-fungal medications can lead to more inflammation, distress, irritation, excoriation, rubbing, maceration, and even secondary infection (21). Regarding this, it is necessary to make an accurate and early diagnosis based on para clinical tests and clinical trials data. Therefore, the aim of the current study was to investigate the prevalence of vulvovaginal infections and characterize the *Candida* species isolated from patients with vulvovaginitis using three different tests.

Materials and Methods

Participants

The sample size of this study was determined using randomized block design; as a

result, 105 samples were examined. First, 1100 patients who had referred to gynecology clinics for various problems were evaluated for 7 months. Among these patients, 450 ones had complaints about genital infections. Based on the patients' history and vaginal discharge characteristics, 280 patients were entered into the study and 105 females were diagnosed with *Candida* infection using serological diagnosis.

This descriptive study was conducted on 105 females referring to the Gynecology Clinics of Shahid Faghihi, Zeynabieh Hospitals, and Hor-e-Riahi Health Center affiliated to Shiraz University of Medical Sciences, Iran during March to September of 2009. Health centers were selected based on their geographical location in the Northern, central, and Southern regions of the city.

Inclusion & exclusion criteria

The inclusion criteria of the current study were as follows: 1) being married; 2) being at reproductive age, 3) not having vaginal bleeding; 4) not suffering from any known chronic diseases such as those weakening the immune system; 5) not having used herbal or chemical drugs for treatment of genital infection in the recent 2 weeks; 6) being diagnosed with *Candida* vaginitis based on direct test and fungal culture; 7) not having undergone vaginal douching within the past 48 h; 8) not having used broad-spectrum antibiotics in the recent 2 weeks; and 9) not having used vaginal creams or suppositories within the past 48 h. On the other hand, the exclusion criteria were negative fungal culture on Sabouraud dextrose agar medium.

Research process

In the first stage, 1100 patients with gynecological problems were visited and 450 females were diagnosed to have genital infections. In the second stage, the patients with *Candida* vaginitis symptoms (itching, discharge, color, ...) were selected through purposive sampling and were entered into the study after signing written informed consents (N=280). In the last stage, 105 patients were diagnosed with candidiasis using three different tests. It should be mentioned that all the samples were collected from the posterior fornix region which contains the highest amount of discharge.

Data collection

The data were collected using a form including the following sections. The first part consisted of the patients' demographic characteristics including age, occupation, marriage age, and education level. The second part of the questionnaire inquired the history of infection, drug consumption, recurrence, and the third part investigated the features of the recent infection, including the intensity of itching, burn, color of discharges, etc.

All stages of research were taken by the researchers and demographic data were collected from patient's medical history.

In order to collect the samples, first, the patient lay in lithotomy position and the smell, color, volume, and other features of vaginal discharges were examined. Then, the samples were collected using speculum and sterile swab. Overall, two samples were obtained from each patient. The first sample was directly put on the lam for preparation of smear and staining by methylene blue method. On the other hand, the second sample was used for culturing on Sabouraud dextrose agar medium in sterile conditions. After sampling, the samples were daily transferred to the laboratory in order to determine the type of fungus.

In order to determine the intensity of infection (the number of fungal colonies) and differentiate *Candida* species, the sterile swabs were dragged on the culture media in a zigzag pattern. Afterwards, the characteristics of the samples, such as number and date, were recorded on the plates containing the culture media. These plates were transferred to the mycology laboratory of School of Medicine at the end of the work shift and were kept in the incubator at 30°C for 24-48 h. Thereafter, in case of colony growth, the colonies were counted, purified by passages, and kept at sterile distilled water at -20°C. In this study, fungal species were identified using germ tube test, CHROMagar medium, and chlamyospore formation by an expert in the School of Medicine laboratory.

Germ tube test

This test is the gold standard method for *C. albicans* diagnosis. In this method, the intended yeast is inoculated in human or rabbit serum diluted by normal saline (1:2) and is kept in the

incubator at 37°C for 2-3 h. In case of Germ tube formation, *Candida albicans* was considered. (22).

CHROMagar test

This test aims to differentiate *Candida* species based on the color produced on the media. In this study, fungal species were purified, cultured, and incubated at 30°C for 48 h, using a chromogenic medium. Thereafter, the types of fungi were determined based on the colonies' colors (HiMedia, India). In this study, the following 8 colors were observed: green, light green, green with a blue border, bluish green, purple, blue, white, and white with blue spots. The first 4 colors were all categorized as green and were interpreted as *C. albicans*. Furthermore, blue and white with blue spots were considered as *C. tropicalis*, while pink, purple, and violet were identified as *C. krusei*. In case yeast was observed in chlamyospore test, white was considered as *C. glabrata*. On the other hand, if pseudo-hyphae was detected in this test, this color represented other *C. non-albicans* species (23, 24).

Chlamyospore test

This test is used for identification of *Candida* species. In general, more than 90% of isolated *C. albicans* form chlamyospore on corn meal agar medium. Similar to germ tube formation, this feature is also specific to *C. albicans*. In this study, the yeasts were cultured through a - corn meal agar medium and were kept at 25°C for a week. In addition to chlamyospore, *C. albicans* also form pseudohyphae on this medium. In case pseudohyphae and blastospore are detected, *C. non-albicans* species are identified. On the other hand, growth of yeast cells is likely to be suggestive different yeast species, most probably *C. glabrata* (23, 24).

Statistical analysis

In this study, data analysis was performed using SPSS 16 (Released 2007, SPSS for Windows, Version 16.0., Chicago, SPSS Inc.). The study variables including age, education status, distribution of different types of organisms, *Candida* species, and color of vaginal discharges were presented as frequency and percentage. The data were checked for normality of distribution and their mean and standard

Table 1. Frequency distribution of the type of organisms in vaginal discharges of the women with *Candida* vaginitis based on germ tube test, Chlamyospore test, and CHROMagar test

Kind of tests	Kind of fungi	N (%)	95% confidence interval
Germ tube test	Positive (<i>C. albicans</i>)	74 (70.5)	(L:61.95; U:79.7)
	Negative (<i>C. non-albicans</i>)	31 (29.5)	(L:20.2; U:38.4)
Chlamyospore test	<i>C. albicans</i>	57 (54.3)	(L:44.5; U:64.1)
	Yeast	23 (21.9)	(L:14.15; U:30.5)
	<i>C. non-albicans</i>	25 (23.8)	(L:14.9; U:31.6)
CHROMagar test	<i>C. albicans</i>	70 (23.8)	(L:57.7; U:76.2)
	<i>C. glabrata</i> and other <i>C. non-albicans</i>	23 (21.9)	(L:14.1; U:30.5)
	<i>C. tropicalis</i>	9 (8.6)	(L:2.5; U:13.02)
	<i>C. krusei</i>	3 (2.9)	(L:0.0038; U:6.2)

deviation were presented.

Ethical considerations

This research project was approved (89-3853) by the local Ethics Committee of Shiraz University of Medical Sciences. The participants' written informed consents were obtained. The research proposal No. 3853 was financially supported by Shiraz University of Medical Sciences.

Results

According to the results of the study, the mean age of the study participants was 32±9.49 years. The mean age of the participants' spouses was 37±11.82 years and most of them (41.9%) had an age range of 26-35 years. Furthermore, the majority of the husbands (52.4%) had primary or middle school degrees. The results of the chi-square test revealed a statistically significant relationship between sexual hygiene and spouses' mean age as well as between sexual health and education ($p \leq 0.001$).

The results also demonstrated that most of the study subjects had primary or middle school degrees (N=60, 57.1%) and were homemakers (N=97, 92.4%). The female participants had experienced 0-8 pregnancies (Mean=2±2.62). As the results demonstrated, the disease showed no significant relationship with occupation, education status, and number of pregnancies ($P > 0.05$).

Additionally, the prevalence of *Candida* vaginitis was found to be 9.3%. *Candida* infection was shown to have the highest incidence within the age of 26-35. Based on the results of germ tube test, 74 patients (70.5%) were diagnosed with *C. albicans* (positive germ tube), while 31 patients (29.5%) were recognized to suffer from *C. non-albicans* (negative germ tube). In the present

study, chlamyospore and yeast were detected on 54.3% and 21.9% of the corn meal agar media, respectively.

In the 105 sample undergoing CHROMagar test in this study, 8 colors were observed. The first 4 colors were all categorized as green and were interpreted as *C. albicans*, which entailed 66.6% of the cases and other colors were considered as *C. tropicalis* and *C. krusei*. On the other hand, if pseudohyphae was detected, white represented other *C. non-albicans* species. In methylene blue staining method, fungal agents were detected in form of yeast and pseudomycelium.

Discussion

According to the results, the highest proportion of infection was detected in the age range of 26-35 years. Based on the study by Asadi (1994), this might be due to higher sexual activity, hormonal physiological changes, and utilization of various contraceptive methods in this age group. In Asadi's study, the mean age of the study subjects was 31 years. The study of khorsandy (1994) was also examined a broader age group, 18-45 year-old women (25, 26).

In the present study, the prevalence of *Candida* vaginitis was found to be 9.3%. Similarly, Shatti (1995) conducted a study in Shiraz to determine females' health behaviors related to vaginal infections and reported the prevalence of *Candida* vaginitis to be 10.3% (27). This low prevalence might be due to the improvement of women's health conditions in the recent years, complete treatment of fungal infections, and increase in the prevalence of non-fungal infections.

In the research Ozcan conducted in Turkey in 2005, the prevalence of vaginitis was

demonstrated to be 35.7% and 16% of the patients were found to suffer from *Candida* vaginitis (28). However, a study in Zanjan reported the prevalence of *Candida* infection to be 4.8% which is less than the results of the current study (29). This difference in the reported prevalence rates might be due to the difference in geographical conditions and study population which consisted of the randomly selected patients who had referred for family planning services and had no complaints about the disease.

In another study in Nigeria conducted on 200 subjects, the prevalence of *C. albicans* and *non-albicans* candidiasis was 6.5% and 7.5%, respectively. Apparently, these results are lower, compared to those of the current study, which may be due to the differences in the study population. The study population in Nigeria was an academic community who may be attributed with adequate knowledge and good personal hygiene (30).

Based on the results of germ tube test, 74 patients (70.5%) were diagnosed with *C. albicans* (positive germ tube). In a study conducted by Aali et al. (1997), *C. albicans* and *C non-albicans* species comprised 75% and 25% of the samples, respectively, which is consistent with the results of the present study (31).

In the current study, chlamyospore and yeast were detected on 54.3% and 21.9% of the corn meal agar media, respectively. Considering the fact that *C. glabrata* is the only species which cannot form pseudomycelium on this medium, the existence of *C. glabrata* can be already assumed. Moreover, there are other species which have been recently introduced as the cause of *Candida* vaginitis; however, they cannot be identified through chlamyospore method.

The results of CHROMagar test demonstrated that *C. albicans* comprised 66.6% of the cases. Although CHROMagar method has been used for identification of *Candida species* for years, it has several limitations. Therefore, diagnosis of species is not highly reliable. In the study conducted by Mohanty, 50%, 35%, 10.8%, and 3% of the cases were caused by *C. glabrata*, *C. albicans*, *C. tropicalis*, and *C. krusei*, respectively (32). Richter and colleagues' study also showed that 80-90% of the cases were induced by *C. albicans* and 10-20% of them were caused by other *Candida* species (33).

Pakshir et al. also conducted a study in Shiraz

and reported that *C. albicans* accounted for 78.75% of the cases, while 21.25% of them were caused by *C. non-albicans*, including *C. glabrata*, *C. krusei*, and *C. tropicalis* (34). Besides, in a study by Gregory (2005), 81% of the cases were caused by *C. albicans* and 19% were diagnosed with *C. non-albicans*, including *C. glabrata*, *C. parapsilosis*, *C. guilliermondii*, and *C. krusei* (35).

A strength of this study was comparison of three diagnostic tests for identifying *Candida* species. Moreover, the fact that the samples were taken by the researcher during seven months increased the accuracy and uniformity of the samples. Besides, the use of a relatively large population was another strength of the current study. One of the research practical limitation was that the sampling was performed by just one sampler, which made the process so long.

It is suggested to carry out the same research with a larger population. In addition, conducting the same study on pregnant females can be helpful in the improvement of prenatal care.

Conclusion

According to the results of the study, *C. albicans* was the most common species leading to the Vulvovaginitis in patients with vaginal candidiasis. Due to inaccuracy of clinical diagnosis of *Candida* vaginitis, it is recommended to use reliable para clinical methods such as fungal culture which is confirmed as a standard diagnostic method, particularly in recurrent and chronic conditions.

Acknowledgements

This study was financially supported by the Research Vice-chancellor of Shiraz University of Medical Sciences (Grant No. 3853). Hereby, the researchers extend their gratitude to the chiefs of Shahid Faghihi, Zeynabiyeh hospitals, and Hor-e-Riahi Health Center for their cooperation.

Conflicts of interest

The authors declare no conflicts of interest.

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