

Full Length Research Paper

Prevalence of vaginal candidiasis infection in diabetic women

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Vulvovaginal Candidiasis (VVC) is a female genital system infection that occurs due to *Candida* species. It is estimated that as many as 75% of women experience at least one episode of Vulvovaginal Candidiasis during the lifetime. The aim of this study is to determine the prevalence rate of Vaginal Candidiasis infection in diabetic women referred to Kermanshah Diabetes Research Center (KDRC), Iran, in 2010. This descriptive – analytic study was performed on 100 diabetic women referred to Kermanshah Diabetes Research Center in 2010. All specimens were examined under direct microscopy and cultured on sabouraud dextrose agar (SDA). Complimentary tests such as germ tube test and sugar assimilation test (API) were carried out to differentiate the *Candida* species. Minimum inhibitory concentration (MIC) test was performed for positive cases. Patients' information was collected by a questionnaire. Statistical analysis was performed using the Chi-square test. Of 100 samples under experiment, 12% (12 cases) in direct microscopy test and 20% (20 cases) by cultivation on SDA were infected to vaginal candidiasis. The frequencies of the isolated *Candida* species include *Candida albicans* with 62/5% (20 cases), *Candida glabrata* with 18/7% (6 cases), *Candida tropicalis* with 9/4% (3 cases) and *Candida parapsilosis* with 9/4% (3 cases). Vulvovaginal candidiasis was more prevalent in women without blood glucose level control than ones with blood glucose level control. *C. albicans* was, by far, the most predominant yeast isolate. The culture of vaginal discharge should be warranted because culture technique is more sensitive than direct smear.

Key words: Vaginal candidiasis, women and diabetes.

INTRODUCTION

Diabetes mellitus is a chronic, insidious disease that can affect any organ of the body. One of the problems associated with this condition is infection (Malazy et al., 2007). Patients with diabetes mellitus are at increased risk of vulvovaginal candidiasis (Goswami et al., 2006). Vulvovaginal candidiasis is the second most frequent infection of the female genital tract (Sobel, 1985; Grigoriou et al., 2006). Up to 75% of all women will

experience fungal vulvovaginitis at some point in the lives, and approximately 40 to 50% will experience a second episode of this disease (Ferrer, 2000; Moreira and Paula, 2006). One cause of recurrent VVC is hyperglycemia.

Candida infection in the vagina can cause a smelly, thick, white–yellow discharge that might be accompanied by itching, burning and swelling. It can also make walking, urinating or sex very painful (Bohanon, 1998). Since the symptoms of vaginal candidosis are not specific to the infection, diagnosis cannot be made solely on the basis of history and physical examination. But the clinical diagnosis of vaginal candidiasis should always be

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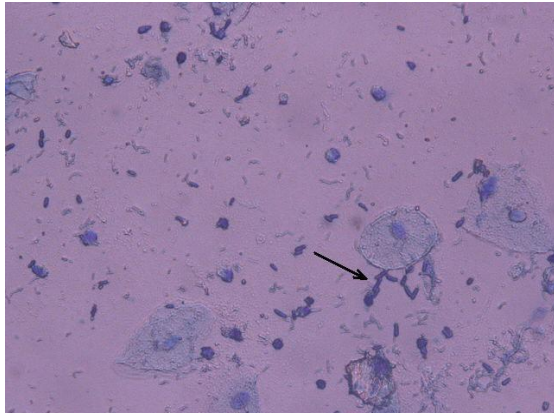


Figure 1. Pseudohyphae identified on microscopic test.



Figure 2. The growth of cells *Candida* on Sabouraud Dextrose Agar.



Figure 3. Germ tube identified on microscopic test.

50% of patients with culture positive symptomatic vaginal candidosis will have negative microscopy. Thus, although routine cultures are not necessary if microscopy is positive, vaginal culture should be done in symptomatic women with negative microscopy (Sobel, 2007).

Candida albicans is the most common species isolated in such an infection in diabetics as well as in non-diabetics. Recently, vulvo-vaginal infection with non *C. albicans* species has been reported with increasing frequency in non-diabetic groups, possibly due to widespread and empirical use of antifungal drugs (Goswami et al., 2000; Spinillo et al., 1995; Sobel et al., 1998). In addition to diabetes, other risk factors for recurrent vulvovaginal candidosis are genetic, pregnancy, the use of oral contraceptives or antibiotics, immune disorders, behavioural factors such as sexual activity and HIV (Sobel, 2007). The aim of this study is to determine the prevalence rate of vulvovaginal candidiasis in diabetic women referred to Kermanshah Diabetes Research Center (KDRC) (Iran, 2010).

MATERIALS AND METHODS

This descriptive-analytic study was performed on 100 diabetic women referred to Kermanshah Diabetes Research Center (KDRC). According to world health organization (WHO), diabetic affliction criterion was fasting blood sugar (FBS) higher than 140 mg/dl in two separate times. We administered a questionnaire to obtain information about: age, occupation, education, symptoms, type of diabetes mellitus, duration of diabetes mellitus, FBS and glycosylated hemoglobin (HbA_{1c}).

The clinical symptoms were not seen on all of the patients. Two sterile cotton-tipped swabs were used to collect discharge from high vagina and sent it to the laboratory without delay. One swab was used to determine the presence of yeast by methyleneblue staining in direct microscopy, while the other was used for fungal culture (culture on sabouraud's dextrose agar supplemented with 50 mg chloramphenicol).

The diagnosis of VVC was based on pseudohyphae identified by microscopic examination and on *Candida* growth on high vaginal swab culture. Isolated strains were identified using the germ tube test and API system 20C AUX (Biomérieux, France) (Figure 1). The susceptibility in vitro to Fluconazole was performed by the National Committee for Clinical Laboratory Standard (NCCLS) (1997) document M27-A. Cells grown on Sabouraud's dextrose agar plates were re-suspended in Sabouraud's dextrose broth so that they can attained a turbidity equivalent of 0.5 McFarland standard (Figure 2). Serial dilution was gained from 64 to 0.125 µg/ml of Fluconazole. A tube was used as a positive control (without antibiotic). The apertures of the burets for growing the fungus were viewed by bare-eyes after 24 h (Figure 3). Anything which caused disdiaphaneity was considered as a resistance against that density. MIC for Fluconazole was defined as the first concentration at which no growth was detected (Goswami et al., 2006; Goswami et al., 2000). Statistical analysis was performed using the Chi-square test and a p-value < 0/05 was considered as significant.

RESULTS

100 diabetic women referred to KDRC were eligible for this study. Thier age varied from 25 to 75 years (51± 9)

confirmed by laboratory diagnosis. Unfortunately, up to

Table 1. Frequency of different *Candida* species isolated from diabetic women referred to Kermanshah Diabetes Research Center (KDRC), Iran in 2010.

<i>Candida</i> species	Positive case (n)	Percentage
<i>C. albicans</i>	20	62/5
<i>C. glabrata</i>	6	18/7
<i>C. tropicalis</i>	3	9/4
<i>C. parapsilosis</i>	3	9/4
Total	32	100

with mean fasting blood sugar level 191 ± 67 mg/dl, and their mean duration of diabetes mellitus was 12 ± 6 years. The duration of diabetes in 50% of them was ≤ 7 and in remaining 50% was > 7 years. 2% of the patients had type I diabetes mellitus and 98% of them type II. Mean glycosylated hemoglobin level in these patients was $7/3 \pm 2$. 58% of the patients had clinical symptoms such as burning, itching and discharge and 42% of them had no symptoms.

Regarding educational level, 86% of our patients were illiterate or had a high school degree, and 14% were college graduates. 90% of patients were housewives, and the rest were working women. The prevalence rate of vaginal candidiasis infection according to direct test and mixture culture reported 12 and 20%, respectively. The species of *Candida* isolated in this patients consisted of *C. albicans* with 62/5% (20 cases), *C. glabrata* with 18/7% (6 cases), *Candida tropicalis* with 9/4% (3 cases), and *Candida parapsilosis* with 9/4% (3 cases), which *C. albicans* was the most predominant yeast isolate (Table 1). In the present study, even though we did not find a significant statistical difference between vulvovaginal candidiasis and occupation, education, symptoms, duration of diabetes mellitus and glycosylated hemoglobin (HbA_{1c}), we found a significant statistical difference between vulvovaginal candidiasis and age, type of diabetes mellitus and fasting blood sugar (FBS) (Table 2). Results of the *in vitro* fungal susceptibility test showed that there existed no significant difference in the Fluconazole susceptibility between *C. albicans* and non-*C. albicans* candidiasis ($P = 1/000$) (Table 3).

DISCUSSION

Although it is commonly believed that vulvovaginal candidiasis in most of the diabetic women is more prevalent than the non-diabetic ones, its conservative relation is not yet known (Reed, 1992; Sobel et al., 1998). Vaginal yeast infection is usually diagnosed on the basis of clinical symptoms, direct microscopic examination and vaginal culture. The microscopic examination of the clinical material is rapidly performed and may identify the presumptive etiologic agent, but vaginal culture is indispensable to confirm the diagnosis (Grigoriou et al.,

2006; Wentworth, 1998). Qingkai et al. (2010) found only 0.3% (1 case) pseudohypha by microscopic examination. Grigoriou et al. (2006) strongly believed that diagnosis must be made thorough vaginal cultures. In our study, on the basis of microscopic examination and the vaginal discharge culture we had 12 and 20% prevalence rate, respectively. Based on this study and also similar studies, it could be said the discharge culture is more sensitive than the direct microscopy test (Figure 4).

Different studies indicated that vulvovaginal candidiasis infection is more common in women with diabetes than in the normal population (Malazy et al., 2007; Scudamore et al., 1992; Sobel, 1993). The prevalence rate ranged from around 7 to more than 50% (Bohanon, 1998; Davis, 1969; Malazy et al., 2007), and most of which was attributed to *C. albicans* (Duerr et al., 1997; Malazy et al., 2007; Otero et al., 1998). Goswami et al. (2006) reported the prevalence rate of 46% in 78 diabetic women. Peer et al. (1993) reported the prevalence rate of the vulvovaginal candidiasis infection 24% in 111 diabetic women. These statistics show that different prevalence rates of vulvovaginal candidiasis in diabetic women are seen. Likely reasons for this difference could be attributed to lack of control on the blood sugar, and also to the use of unsuitable antifungal agents.

Nowadays, because of prevalence of the vulvovaginal candidiasis infection and drug resistance of some *Candida* species, it is necessary to determine disease causing factors separated from the waste. In this study, which was similar to other studies like Antony et al. (2009), Corsello et al. (2003), Grigoriou et al. (2006), Maccato and Kaufman (1991) and Peer et al. (1993), the most separated species from the patients was *C. albicans*. The first step in establishing a yeast infection is bonding to the vaginal mucosa. It seems that *C. albicans* is more adhesive than other non-*C. albicans* species. This could be considered as one of the likely reasons that this species are predominant rather than non-*C. albicans* species (Grigoriou et al., 2006; Maccato and Kaufman, 1991) unlike other studies on the prevalence of non-*C. albicans* species that has increasing species (Duerr et al., 1997; Goswami et al., 2006; Otero et al., 1998; Sobel et al., 1998). A high rate of prevalence of non-*C. albicans* species was not achieved in our study. Goswami et al. (2000) reported the prevalence rate of non-*C. albicans*

Table 2. Predisposing factors for infection in diabetic women referred to Kermanshah Diabetes Research Center (KDRC), Iran in 2010.

Predisposing factors	Positive case (n)	Percentage	P value
Age :			
≤ 40 years	4	20	0/02
> 40 years	16	80	
Occupation :			
Working	7	30	0/248
Housewife	13	70	
Education :			
College	3	20	0/023
illiterate, high school	17	80	
Symptoms :			
Yes	14	7	0/413
No	24	12	
Duration of diabetes mellitus :			
≤ 7 years	12	60	0/317
> 7 years	8	40	
HbA1C :			
< 7%	12	60	0/317
≥ 7%	8	40	
FBS :			
< 200 mg/dl	4	20	0/041
≥ 200 mg/dl	16	80	
Diabetes mellitus :			
Type I	2	10	0/004
Type II	18	90	

Table 3. Antifungal susceptibilities of the selected *C. albicans* and non-*C. albicans* isolates.

<i>Candida</i> species(n)*	Antifungal agent (Fluconazole)	
	Mean MIC	P value
<i>C. albicans</i> (10)	0.60	1/000
Non- <i>C. albicans</i> (10)	0.57	1/000

* Number of selected isolate for the test.

species 39% with the predominant species *C. glabrata* in diabetic women. Sobel and Malazy (1998, 2007) stated the probable causes of higher non-*C. albicans* species: the short duration of use for oral or local anti-*Candida* regimens; the widespread use of over-the-counter antifungal agents. Similar to the study by Malazy et al. (2007), a significant statistical difference not found in this

study. Instead, a or relationship between positive vaginal candida culture and occupation, symptoms, duration of diabetes mellitus and glycosylated hemoglobin was significant statistical difference between positive vaginal *Candida* culture and fasting blood sugar was discovered Bohanon (1998) stated that the main causes of this state of affairs are hyperglycemia. Increased glucose levels in

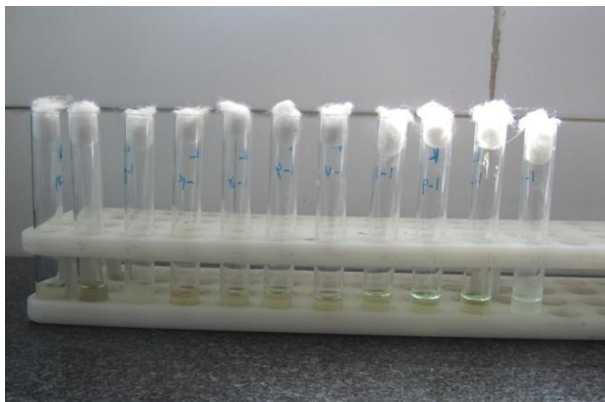


Figure 4. Dilloution identified on MIC test.

genital tissues enhance yeast adhesion and growth. Vaginal epithelial cells bind to *Candida* with greater propensity in diabetic patients than in non-diabetic patients. Furthermore the control of blood glucose levels plays an important role in controlling vaginal *Candida* infection in diabetic women. In the present study, also a significant statistical difference between positive vaginal *Candida* culture and type of diabetes mellitus was found. Similar to the findings of the study carried out by Malazy (2007), in this study, it was found that most of the individuals with vaginal candidiasis infection suffered from type II diabetes, solely due to the age of patients. The mean age of them was 51 years and they were mainly categorized into an age range over 40 years. While type I diabetes occur before 30, it should also be noted that the prevalence rate of vaginal candidiasis infection occurs during pregnancy.

The symptoms often include burring, itching and discharge which have been proved by many relevant studies (Peer et al., 1993). In addition, Peer et al. (1993) showed that 25/4% of infected people with vaginal candidiasis infection did not present any clinical symptom. It can be concluded from the above points that vaginal candidiasis infection is not always accompanied by sever detectable symptoms and sometimes it does not demonstrate any symptom and sometimes it shows very mild symptoms.

A significant statistical difference between vaginal candidiasis infection and education was found in this study, which could be attributed to the observance of genital hygiene and low level of glucose in diet. We did not find a significant statistical difference between infectious vaginitis and glycosylated hemoglobin, because acute infections such as vaginitis often occur during the hyperglycemic state, but glycosylated hemoglobin reflects the mean blood glucose level over the previous 3 months (Malazy et al., 2007).

In this study, Goswami (2006) showed no significant difference in the fluconazole susceptibility between *C. albicans* and non-*C. albicans* candidiasis. In addition, in

the current study, we did not find a significant statistical difference in the Fluconazole susceptibility between *C. albicans* and non-*C. albicans* candidiasis. Prescribing general drug for a long duration causes a drug resistance. Therefore, awareness of the drug resistance *Candida* separated from vaginitis against the general anti-*Candida* drug is needed to cure involuting cases properly.

Conclusion

The present study involved only 100 patients and majority of them had type II diabetes mellitus. There is need to perform similar study in large number and for a longer duration. But according to the results in this study and similar ones, diabetic was introduced as one of the risk factors of VVC; it is recommended that these patients observe blood sugar control and hygienic issues.

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