



Species-specific prevalence of vaginal candidiasis with type 1 and type 2 diabetes mellitus among women in Sana'a city

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ABSTRACT

Vaginal candidiasis (VC) is a fungal or yeast infection of the vulva and/or vagina. It causes a smelly, thick, white-yellow discharge that might be accompanied by itching, burning and swelling. Vaginal candidiasis is the second most frequent infection of the female genital tract. 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 patients with diabetes mellitus are at increased risk of vaginal candidiasis. The purpose of this study was to determine the prevalence and species distribution of *Candida* species isolated from vaginal candidiasis infection in diabetic and non-diabetic women in Sana'a City. This descriptive – analytic study was performed on 150 diabetic women referred to Al-Thaourah and AL-Gmohore hospitals in Sana'a City. All specimens were diagnosed or examined under direct microscopy and cultured on Sabouraud dextrose agar (SDA) or (YEPDA) media. Complimentary tests such as germ tube test, corn meal agar media, CHROM agar test and sugar fermentation test were carried out to differentiate between the *Candida* species. Minimum inhibitory concentration (MIC) test was performed for positive cases. Patient's clinical history information was collected by a questionnaire. Statistical analysis was performed using the Chi-square test. Of 150 samples tested under all biochemical experiments and culture methods, 62 out of 150 with percentage 42% were infected to vaginal candidiasis. The frequencies of the isolated *Candida* species include *Candida albicans* 33 species with 53%, *Candida glabrata* with 19 species with 31%, *Candida tropicalis* 6 species with 10% and *Candida krusei* 4 species with 6%. Vaginal candidiasis was more prevalent in women infected with diabetes mellitus than ones with non-diabetes mellitus women. 11 patients out of 150 patients with percentage 8% of the patients had type I diabetes mellitus and 51 patients out of 150 with percentage 34% of them type II with ratio 18:82 from totally of 62 out of 150 with 42% vaginal candidiasis patients had infected. *C. albicans* was, by far, the most predominant yeast isolates. The culture technique of vaginal discharge not be warranted so we used the microscopic and biochemical tests because are more sensitive in identification of *Candida* species than culture methods. But vaginal culture is indispensable to confirm the diagnosis by microscopic examination.

Keywords: Vaginal candidiasis ; diabetes mellitus; among women.

INTRODUCTION

Diabetes mellitus is a chronic, insidious disease that can affect any organ of the body. Hyperglycemia is the major cause of increased susceptibility of diabetic patients to vulvovaginal candidiasis. Increased glucose levels in genital tissues enhance yeast adhesion and growth. Vaginal epithelial cells bind to *Candida albicans* with greater propensity in diabetic patients than in non-diabetic patients, regardless of whether the patients are

premenopausal, postmenopausal, or pregnant [1]. Patients with diabetes mellitus are at increased risk of vaginal candidiasis [2]. Vaginal candidiasis is the second most frequent infection of the female genital tract [3, 4]. Up to 75% of all women will experience fungal vaginitis at some point in the lives, and approximately 40 to 50% will experience a second episode of this disease [5, 6]. One cause of recurrent VC 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 [7]. Since the symptoms of vaginal candidiasis 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 50% of patients with culture positive symptomatic vaginal candidiasis 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 [8]. *Candida albicans* is the most common species isolated in such an infection in diabetics as well as in non- diabetics. Recently, 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 [9, 10, 11]. In addition to diabetes, other risk factors for recurrent vaginal candidiasis are genetic, pregnancy, immune disorders, behavioral factors such as sexual activity, the use high-estrogen-dose oral contraceptives, obesity, drug addiction and HIV [12,13] The aim of this study is to determine the prevalence rate of vaginal candidiasis in diabetic women in Sana'a City.

EXPERIMENTAL SECTION

This descriptive–analytic study was performed on 150 diabetic women in Sana'a City. 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, and duration of diabetes mellitus, FBS and glycosylated hemoglobin (HbA1C). 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. The swab was used to fungal culture (culture on Sabouraud's dextrose broth supplemented with 50 mg chloramphenicol) then incubation at 37°C the apertures of the burettes for growing the fungus were viewed by bare-edges after 24 - 48 hours [29]. The positive sample it was determine the presence of yeast by methylene blue staining in direct microscopy (Figure 2). On the other hand, pure culture on SDA media it was prepared for other diagnosis (Figure 1). The diagnosis of VC was based on pseudohyphae and chlamydo spores identified by microscopic examination and on candida growth on high vaginal swab culture. Isolated strains were identified using the germ tube test (Figure 3). CHROM agar test and sugar fermentation test. The susceptibility in vitro to Fluconazole was performed by the National Committee for Clinical Laboratory Standard (NCCLS) (1997) document M27-A. Serial dilution was gained from 64 to 0.125 µg/ml of Fluconazole. A tube was used as a positive control (without antibiotic). Anything which caused disdiffidently was considered as a resistance against that density. MIC for Fluconazole was defined as the first concentration at which no growth was detected. Statistical analysis was performed using the Chi-square test and a p-value < 0/05 was considered as significant.

RESULTS AND DISCUSSION

150 diabetic women were eligible for this study. Their age varied from 40 to 75 years (57±9) 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. 11 case out of 150 cases with percentage 8% of the patients had type I diabetes mellitus and 51 cases out of 150 with percentage 34% of them type II with ratio 18:82 from totally of 62 out of 150 with 42% vaginal candidiasis patients had infected. . There seems to be a significant link between hyperglycemia and vaginal candidiasis.

Mean glycosylated hemoglobin level in these patients was 7/3 ± 2. 58% of the patients had clinical symptoms such as burning, itching and discharge and 42% of them had no symptoms.

Regarding educational level, 76% of our patients were illiterate or had a high school degree, and 24% were college graduates. 81% of patients were housewives, and 19% were working women. The prevalence rate of vaginal candidiasis patients according to culture on Sabourauds dextrose agar (Figure 1), direct microscopic gram staining

(Figure 2), biochemical tests and mixture culture reported 62 out of 150 patients with 42%. While 98 out of 150 patients were no infected with vaginal candidiasis.

Table 1. Frequency of different *Candida* species isolated from diabetic women

<i>Candida</i> species	Positive case(n)	Percentage
<i>C. albicans</i>	33	53%
<i>C. glabrata</i>	19	31%
<i>C. tropicalis</i>	6	10%
<i>C. krusei</i>	4	6%
Total	62	100

The species of *Candida* isolated in these patients consisted of *C. albicans* 33 species with 53%, *C. Glabrata* 19 species with 31%, *Candida tropicalis* 6 species with 10%, and *Candida krieuse* 4 species with 6% which *C. albicans* was the most predominant yeast isolate (Table1). Identification of *Candida spp.* was performed by identified *Candida albicans* on cornmeal agar cultures (Figure 4) and on germ tube test (Figure 3).

In the present study, we found a significant statistical difference between vaginal candidiasis and occupation, education, but there were no significant differences between the frequency of itching (55.9 vs. 56.7%), vaginal discharge (63.8 vs. 69.0%), dyspareunia (25.0 vs. 20.0%), and percentage yeast positivity (67.5 vs. 54.7%) duration of diabetes mellitus, Also women with abnormal HbA1c level, were almost three times more likely to be colonized with *Candida* than those with normal HbA1c level, but this association was not statistically. On the other hand, we found a significant statistical difference between vaginal candidiasis and age, type of diabetes mellitus and fasting blood sugar (FBS) (Table 2).

Table 2. Predisposing factors for vaginal candidiasis infection in diabetic women

Predisposing factors	Positive case(n)	Percentage (%)	P value
Age:			
≤ 40years	14	23	0/02
> 40years	48	77	
Occupation:			
Working	12	19	0/004
Housewife	50	81	
Education:			
College	15	24	0/004
illiterate, highschool	47	76	
Symptoms:			
Yes	19	30	0/414
No	43	70	
Duration of diabetes mellitus:			
≤ 7years	39	63	0/319
> 7years	23	37	
HbA1C :			
< 7% Normal	24	39	
≥ 7% Abnormal	38	61	0/318
FBS:			
< 200mg/dl	12	19	0/004
≥ 200mg/dl	50	81	
Diabetes mellitus:			
Type I	11	8	0/003
Type II	51		18.82

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). We found strong, statistically significant associations between vaginal *Candida* colonization and diabetic women aged 41 years and older in our study were 48 with 77%. Although, it is commonly believed that vaginal candidiasis in most of the diabetic women is more prevalent than the non-diabetic ones, its conservative relation is one of the concerning facts is that about 25-50 percent of healthy women have yeast infestation in the vaginal area. In a healthy woman, the acidic condition in the vaginal area controls the growth and division of yeast.

Figure 1-Candida albicans on Sabouraud - Dextrose agar at 48h at 37°C



Figure 2- Photomicrograph of budding blastoconidia in Candida species



Figure 3- Germ tube formation in human serum $\times 40$

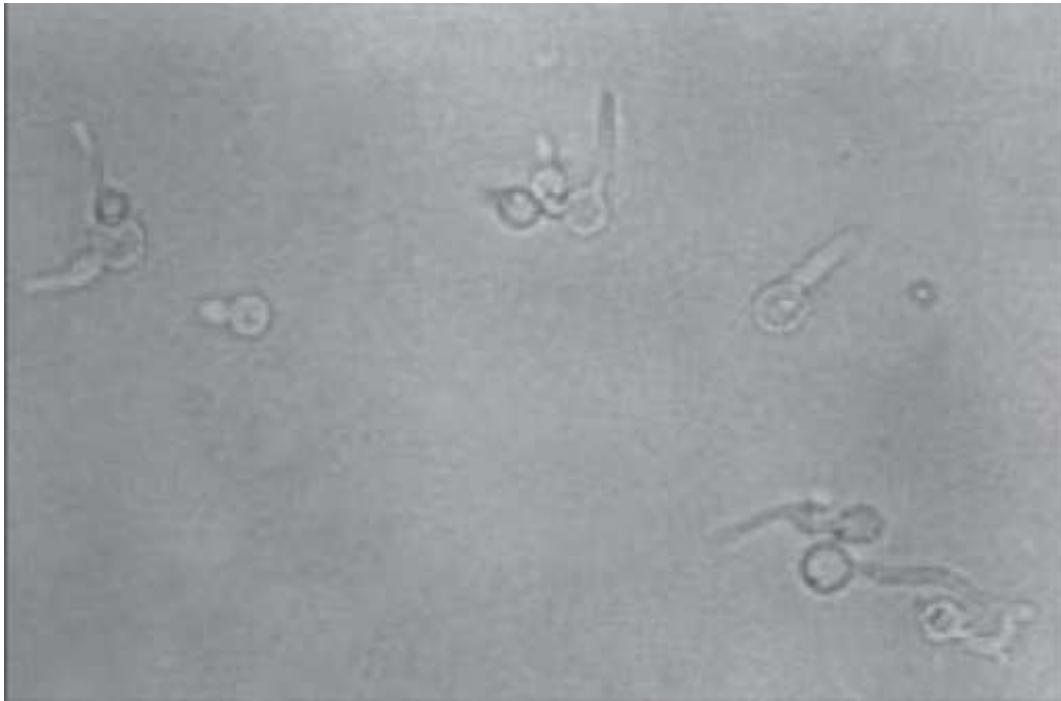


Figure 4- Chlamydospore formation in corn meal broth media with at 24 hours in diabetic women cases $\times 40$

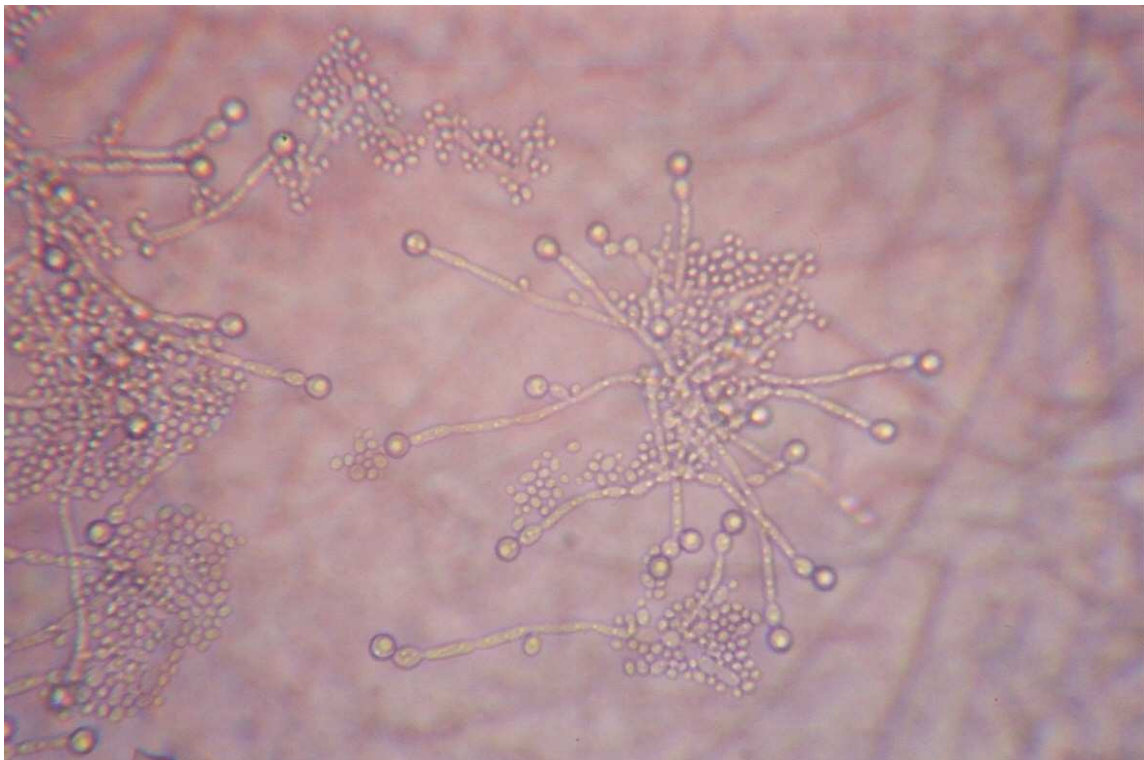


Table 3. Antifungal susceptibilities of the selected *C. albicans* and non-*C. albicans* isolates

Antifungal agent (Fluconazole)		
<i>Candida</i> species(n)*	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.

*MIC: The minimum inhibitory concentration

CONCLUSION

On the contrary, the vaginal secretions of diabetic women shows higher amount of glucose, which in turn helps in promoting the division of yeast. Over a period of time, the woman with diabetes develops yeast infections [14, 15]. Along with pregnancy, use of broad-spectrum antibiotics, high-estrogen-dose oral contraceptives, obesity, and drug addiction as other causes.

Vaginal yeast infection is usually diagnosed on the basis of clinical symptoms, direct microscopic examination and vaginal discharge 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 by microscopic examination [16]. Strongly believed that diagnosis must be made thorough vaginal cultures with microscopic and biochemical examination.

Different studies indicated that vaginal candidiasis infection is more common in women with diabetes than in the normal population [17, 18, 19]. The prevalence rate ranged from around 7 to more than 50% [20, 6, 13] and most of which was attributed to *C. albicans* [21, 22]. [9] reported that the prevalence rate of 46% in 78 diabetic women. [23] reported that the prevalence rate of the vaginal candidiasis infection 42% in 111 diabetic women and so these results concordant with our study results.

We reported in our study, the prevalence rate of the vaginal candidiasis infection 62 with 42% out of 150 diabetic women studied. These statistics showed simple different in prevalence rates of vaginal candidiasis in diabetic women with the previous studies. Likely reasons for this result be attributed that diabetes is a known predisposing factor of vulvovaginal candidiasis, primarily because of hyperglycemia-induced alterations, including decreased random motion of neutrophils, chemotaxis, phagocytosis, and microbial killing. In addition, increased glucose levels in genital tissues enhance yeast adhesion and growth. Most study data indicate that overall yeast carriage and infection rates increase in diabetes patients and correlate with degree of glucose control. Thus, good control of blood glucose and treatment with an appropriate antifungal agent are important in the management of vaginal candidal infections in diabetic women.

In this study, which was similar to other studies like [24, 11, 12, 16] the most separated species from the patients was *C. albicans*. In other study, *Candida albicans* is the principal responsible accounting for over 80% of total vulvovaginal candidiasis [25].

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 [26, 27, 11] unlike other studies on the prevalence of non-*C. albicans* species that has increasing species [7, 8, 9]. [10, 12] reported the prevalence rate of non-*C. albicans* species 39% with the predominant species *C. glabrata* in diabetic women. [27] 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 [3, 15] a significant statistical difference not found in this study. Instead, a relationship between positive vaginal *Candida* culture and symptoms, duration of diabetes mellitus and glycosylated hemoglobin but was discovered significant statistical difference between positive vaginal *Candida* culture and fasting blood sugar, education level and occupation. [4] stated that the main causes of this state of affairs are hyperglycemia increased glucose levels. But 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 [27].

In the current study, we did not find a significant statistical difference in the fluconazole susceptibility between *C. albicans* and non-*C. albicans* candidiasis. Although, an antifungal drug is still medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as Cryptococci meningitis, and others but prescribing general drug for a long duration may cause a drug resistance [28]. On the other hand, medication used also to improve blood sugar control includes Metformin (Glucophage), or insulin sensitizers such as Pioglitazone, Rosiglitazone, or Troglitazone [28]. Modest weight loss in women who are overweight may prove beneficial in improving insulin sensitivity and glucose tolerance [29]. Nevertheless, the causing infections of the hair, mucous membranes, nails or skin include *candida* and dermatophyte fungi while the drugs are remain active against fungi like *Candida albicans*, *Aspergillus Niger* [30]. Therefore, awareness of the drug resistance *Candida* separated from vaginitis against the general anti-*Candida* drug is needed to cure involving cases properly.

The present study involved only 150 patients and majority of them had type II diabetes mellitus. But according to the results in this study and similar ones, diabetic was introduced as one of the risk factors of VC in addition other risk factors; it is recommended that these patients observe blood sugar control and hygienic issues. Genital tissues nature definitely enhances 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. In this study also, it was found that most of the women with vaginal candidiasis infection suffered from type II diabetes, mostly due to the age of patients. The mean age of them was 57 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 intercourse sexual and pregnancy. A significant statistical difference between vaginal candidiasis infection and education and occupation was found in this study, which could be attributed to the observance of genital hygiene and low level of glucose in diet. In this study, we showed no significant difference in the fluconazole susceptibility between *C. albicans* and non-*C. albicans* candidiasis so other studies are needed for confirm that.

Acknowledgements

The authors would like to express about their appreciation and deep gratitude to Al-Yemenia University for their humble financial support for this research. The authors are thankful to the Al-Thaourah and AL-Gmohore laboratories for technical support.

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****NOTE**, This paper has been represented as oral presentation in the international conference on medical & health sciences which held in renaissance hotel, Kota Bharu, Kelantan, Malaysia during period from 22 to 24 MAY 2013.