

Prevalence of Vulvovaginal Candidiasis among women with Diabetes mellitus in Ibadan, Oyo State, Nigeria

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Abstract

Background: Diabetes mellitus predisposes to both bacterial and fungal infections, including *Candida species*. Hitherto, *Candida albicans* has been identified as the most common opportunistic pathogen among patients with diabetes mellitus. More recently, *Non-Candida albicans Candida* (NCAC) *species* are increasingly recognized as the cause of candida infections.

Objective: To determine the prevalence of vulvovaginal candidiasis (VVC) as well as the species of *Candida* frequently identified among women with diabetes mellitus in Ibadan, Nigeria.

Methods: A cross-sectional study of 213 women diagnosed with diabetes mellitus was carried out in 2010. Direct microscopy and fungal cultures of high vaginal swabs were done using Sabouraud--Dextrose Agar and Chrom Agar.

Results: The prevalence of VVC among 213 women with diabetic mellitus was 18.8% (40/213). The predominant *Candida species* isolated were *Candida glabrata* (30.0%), *C. albicans* and *C. tropicalis* (17.5%) each and *C. Gulliermondii* (15.0%). Diabetic women had higher rates of moderate and heavy growth of *Candida* density. Twenty-nine (72.5%) patients with candidiasis were symptomatic and the most common symptom was vulval/vaginal itching 48.3% (14/29).

Conclusion: This study put the prevalence rate of VVC among women with diabetes mellitus in Ibadan at 18.8%. The most common *Candida species* isolated was *C. glabrata* and majority of the patients were symptomatic.

Key words: Candidiasis, Diabetes mellitus, Prevalence, Vulvovaginitis, Women

Introduction

Diabetes mellitus is a group of disorders of carbohydrate metabolism or a state of chronic hyperglycemia (i.e. an excessive concentration of glucose in the blood) resulting from impaired metabolism of carbohydrates, as a result of insufficient insulin secretion by the pancreas.^[1,2]

Diabetes mellitus predisposes individuals to bacterial and fungal infections including those caused by *Candida species*.^[3-5]

Candidiasis remains an important clinical problem, primarily among the immune-

compromised population. *Candida albicans* is the most important species of candida in clinical practice but other species have gained increasing clinical importance such as *C. glabrata*, *C. krusei* and *C. dubliniensis*. One of the reasons for the emergence of these other species may be improved scientific methods or increasing use of some antifungal drugs which might have resulted in the emergence of some of these previously harmless organisms as pathogens.^[6,7]

Infection with *Candida species* is more prevalent among patients with diabetes mellitus than the non-diabetic population. In a study of people with diabetes and non-diabetic controls, 31% of the former compared with 5% of the latter had *Candida* infections.^[8] Disseminated candidiasis

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among patients with diabetic mellitus has been described in the literatures and has been associated with decreased serum beta-globulin anti-candidal factor.^[8]

Although diabetes mellitus has long been recognized as a risk factor for vulvo-vaginal candidiasis (VVC), there is paucity of species-specific data in this regard. A Finish study reported a prevalence rate of 55% of vaginal candidiasis among 166 girls aged less than 15 years with diabetes.^[8] The *Candida* species isolated in the study included *C. albicans* (64%), *C. krusei* (12%), *C. glabrata* (3.3%), *Saccharomyces* (4.3%) and other forms of yeasts (7.6%). In India, a prevalence rate of 78% of *Candida* species was reported among 82 women with diabetes mellitus and clinically overt vaginitis.^[10] In South Africa, a 39.9% prevalence of vulvo-vaginal candidiasis was reported in a cohort of 203 women with diabetes mellitus; *C. glabrata* was the commonest specie identified (50%) followed by *C. albicans* (36.1%).^[11] Furthermore, in USA, 28.7% of 101 women with diabetes mellitus were colonized with *Candida* species.^[3] The most common species in that study included *C. albicans* and *C. glabrata*.

In Nigeria, some studies have described the prevalence of VVC among women. The prevalence of VVC in studies among non-pregnant women, ranged from 36.5% to 60%^[12,13] while it ranged from 62.2% to 70% among pregnant women.^[13,14] However, there is paucity of data on the prevalence of VVC among women with diabetes mellitus in Nigeria. One study carried out in Ibadan on the pattern of microbial colonization among patients with diabetes mellitus reported a prevalence of 27%.^[15] Given this high local burden of microbial colonization, this study was carried out to describe the prevalence of VVC among women with diabetes mellitus.

Methods

Study Population

The study was a descriptive cross-sectional survey which was carried out among women diagnosed with diabetes mellitus who attended three clinics in Ibadan, Oyo State, Nigeria, namely: the Department of Medicine, University College Hospital (UCH), Diabetic Clinic, Oyo State Hospital, Ring Road and Our Lady of Apostle (OLA) Catholic Hospital, Oluyoro. A total of 213 consecutive women attending the three different institutions and with the clinical diagnosis of diabetes mellitus, were recruited into the study. These included 114 patients from UCH, 81 from Oyo State Hospital and 18 from OLA, Oluyoro over a one year period between September 2009 and December 2010. Informed consent was obtained from all the respondents and this included the consent to review their medical records. Ethical approval for the study was obtained from the Institutional Review Committee of the University of Ibadan/University College Hospital prior to the commencement of the study. A short questionnaire was administered on all the participants to obtain information regarding age, types of medications, marital status, educational status, duration of diabetic illness, type and severity of illness, recent antibiotic use, sexual history and symptoms of VVC. Women who took antibiotics two weeks prior to the study were excluded.

Sample Collection

For each participant, venous blood was obtained for fasting blood glucose and 2 hours post-prandial blood glucose (2HPP) and glycosylated Haemoglobin A concentration (HbA_{1c}). All the blood samples were transported to the laboratory immediately for processing. Each of the samples for glucose estimation was centrifuged at 3,200 rpm for 5 minutes;

thereafter, plasma was obtained and separated into plain bottles.

Each respondent was also placed in the lithotomy position and a sterile non-lubricated Cuscos speculum was inserted into the vagina. Vaginal secretions from the posterior fornices were collected using two sterile, cotton typed swabs.

Processing of the vaginal secretions

The vaginal specimens were cultured for yeast. The colonies of suspected/confirmed yeasts were then sub-cultured on Chrome agar plate to aid identification. The microscopic examination of vaginal swab specimens was carried out and smears were made from the colonies of grown cultures. Germ tube positive cells showed a tube like extrusion from the parent cell, several millimetres long. The yeast cells grown overnight on Sabouraud agar were removed with a platinum wire loop, smeared thinly in Zig-Zag lines on the cornmeal-Tween agar plate and covered with a sterile cover glass. The closed Petri dish was kept at room temperature for 24-48 hours. It was then observed under low and high power objective for the development of chlamydospore, blastospore and pseudomycelium.

A single colony of yeast was inoculated into peptone water sugar in a McCartney bottle each containing Durham tube. These were incubated at room temperature for 5-7 days and the production of acid and gas was observed. Positive fermentation was indicated by pink-red colouration. Urea agar slope was stabbed with a small portion of the yeast colonies. It was incubated at room temperature and examined daily for 3 -5 days; positive reaction was indicated by change of colour from light orange phenol red to pink.

Species Identification

The identification of *C. albicans* was done using the germ tube test. A straight wire loop was used to inoculate small quantity of yeast cell into 0.5ml of sterile human serum in a test tube. The test tubes were incubated at 37°C for two hours, after which microscopic examination at x10 and x40 objective was done. A Pasteur pipette was used to put a drop of yeast culture on a clean, dry, grease-free slide; this was in turn covered with a cover slip. Germ tube positive cells showed a tube like extrusion from the parent cell, several millimetres long while germ tube negative cells showed a tube like extrusion.

The identification of *Candida* species was done by means of sugar assimilation test using two methods; namely the punch hole method and the impregnation disk method. In the punch hole methods, the melted minimal agar which had been well mixed with 2mls of the suspension of the test organism was poured into a sterile petridish. This was allowed to solidify and the plate was properly dried in an incubator for about an hour. With the aid of sterile puncher, holes were made in the media at a distance of 30mm to each other. Then each hole was well labelled for each sugar solution. The corresponding 20% solutions of sugar: glucose, xylose, maltose, galactose, sucrose, lactose, and cellobiose were used to fill the holes using sterile Pasteur pipette. This was incubated in applicant position (to avoid spilling) for 48 hours at room temperature. The impregnation disk method used disk prepared by impregnating 20% sugar solution each for glucose, xylose, maltose, galactose, sucrose, lactose, and cellobiose. The poured minimal agar plate was well dried at 50°C for 15 minutes and was flooded with the suspension of the test organism. The excess inoculum was drained into the discard jar and the surface of the flooded agar medium was allowed to dry.

Labelled disks for 20% sugars solutions were then placed on the medium at distance of 30mm to each other. This was incubated in application position at room temperature for 48 hours. A milky halo of cultured yeast colonies, corresponding to the diffusion zone of the substances formed around the test disks or holes, indicate assimilation of the sugar.

The data generated were analyzed using SPSS version 19 statistical software.

Results

A total of 213 women with diabetes mellitus were studied. Out of these 213 women, 14 (6.6%) had Type-1 disease while the remaining 199 (93.4%) had Type- 2 diabetes mellitus.

Table I shows the age distribution of the participants. The highest frequency was in the 45-54 years age group (58%), followed by the 55-64 years age group (55%) in descending order; it was lowest in the 15-24 years age group (4%).

Of the 40 women with diabetes mellitus who had candidal growth, 7 (17.5%) had *Candida*

Table II shows that 40 (18.8%) out of 213 women with diabetes were infected with *Candida* species.

Table II: Prevalence of *Candida* species among women with diabetes mellitus in Nigeria

Characteristics	Frequencies	Percentages
Isolation of <i>Candida</i> species (n = 213)		
Yes	40	18.7
No	173	81.3
Identity of <i>Candida</i> species (n = 40)		
<i>Candida albicans</i>	7	17.5
Non- <i>Candida albicans</i> (NCA)/Mixed species	33	82.5
<i>Candida</i> species density (n = 40)		
Light	11	27.5
Moderate/Heavy	29	72.5
Control of Diabetes mellitus (n = 40)		
HbA1c >9.1%	24	60.0
HbA1c <9.1%	16	40.0

albicans while 31 (77.5%) had Non-*Candida albicans* (NCA) species while the remaining 2 (5.0%) had mixed growth of *Candida albicans* and NCA species. Twenty-nine (72.5%) of the 40 women with diabetes mellitus had moderate/heavy growth, while 11(27.5%) had only light growth. Figure 1 shows that *C. glabrata* had the highest isolation rate 12(30.0%). Twenty-nine out of 40 (72.5%) women with *Candida* spp. were symptomatic. As shown in Table III, vulval/ vaginal itching (63.6%) was the most frequent symptom among the 22 women with Non-*Candida albicans* specie infections while itching with whitish discharge was the most common symptom (42.8%) among women with *C. albicans* infections.

Discussion

The prevalence rate of VVC among women with diabetes mellitus in the present study was 18.8% in this study. The prevalence rate was lower than the rates reported in some previous studies.^[9,10,14,16] The variation in the prevalence

Table I: Age distribution and types of Diabetes mellitus among women in Ibadan, Nigeria

Characteristics	Frequencies	Percentages
Age (Years)		
15-24	3	1.4
25-34	6	2.8
35-44	28	13.1
45-54	56	26.3
55-64	54	25.4
65-74	55	25.8
>75	11	5.2
Type of Diabetes mellitus		
Type 1	14	6.6
Type 2	199	93.4

rates of VVC among the various studies compared, could be due to the differences in the age distribution in those studies compared to our study of relatively older population. The increased frequency of VVC in women with diabetes mellitus could be attributed to the fact that, in diabetes mellitus, there is altered host response to infections with resultant increased susceptibility to fungal infections.^[16, 17] The present study showed that women with diabetes mellitus had a frequency of Non-*C. albicans* species of 77.5% with *C. glabrata* contributing the highest proportion of 30.0%. This is in agreement with other studies that reported *C. glabrata* as the predominant species isolated among women with diabetes mellitus and VVC.^[5,18]

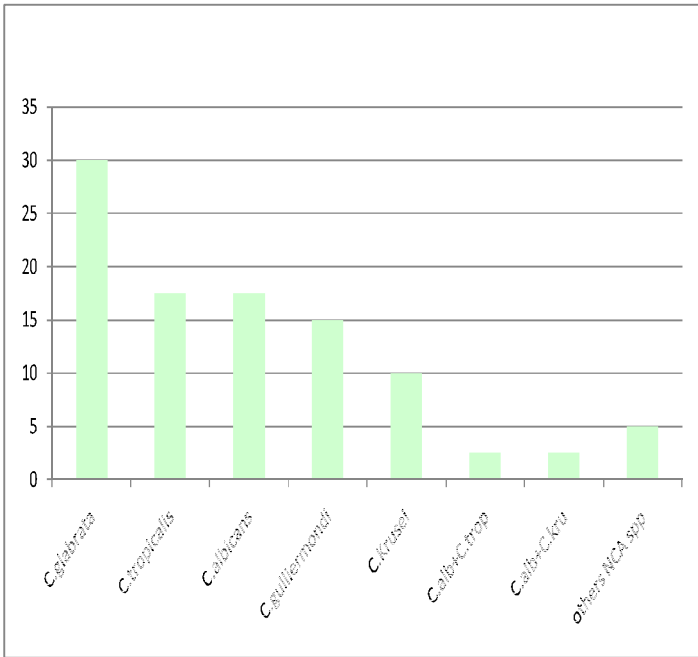


Figure 1: Frequencies of *Candida* species isolated among women with diabetes mellitus and vulvovaginal candidiasis in Ibadan.

Table III: Frequency distribution of *Candida* species and specific symptoms of candidiasis among symptomatic candidal culture- positive diabetic mellitus women (n=29)

Symptoms	Total	Candida species	
		C. albicans	NCAS/Mixed
Vulval/vaginal itching only	14 (48.3)	0 (0.0)	14 (100.0)
Itching with whitish discharge	5 (17.2)	3 (60.0)	2 (40.0)
Whitish discharge only	5 (17.2)	2 (40.0)	3 (60.0)
Burning sensation	2 (6.9)	0 (0.0)	2 (100.0)
Itching with discharge and soreness	3 (10.4)	2 (66.7)	1 (33.3)
Total	29 (100.0)	7 (24.1)	33 (68.1)

NCAS = Non-*Candida albicans* species
Figures in parentheses are percentages

It appears that candidiasis due to NCA species is usually associated with diabetes mellitus and more severe conditions of immunosuppression such as neutropaenia and advanced stages of Acquired Immune Deficiency Syndrome.^[19-21] The cause of the relatively higher prevalence of *C. glabrata* infection in patients with diabetes mellitus is not clear. Meanwhile, it has been documented that factors other than the level of glycaemic control and phagocytic activity of neutrophils might play important roles in determining the predilection of patients with diabetes mellitus to *C. glabrata* infection.^[18]

It was also observed in the present study that, women with diabetes mellitus and VVC due to *C. albicans* had symptoms such as whitish vaginal discharge and vulval itching with or without soreness. On the other hand, VVC due to NCAS mostly experience itching only. However, another study reported comparable clinical symptoms between women with diabetes mellitus infected with *C. glabrata* and *C. albicans*.^[22] The increasing frequency of *C. glabrata* infections among women with diabetes mellitus may have some clinical implications. Studies have reported poor therapeutic

response and innate resistance of *C. glabrata* to the azoles group of anti-fungal drugs among women without diabetes mellitus.^[23-26]

Conclusion

The present study has demonstrated the high prevalence of vulvovaginal candidiasis among women with diabetes mellitus and the prominent role of non-*Candida* species, *C. labrata* in this cohort.

Authors' Contributions: AOE conceived the study and collected the data, OA provided support in the design of the study and supervised the project, OJD wrote the initial manuscript and made significant contribution, OKS provided statistical support, FAA granted access to the clinic where the patients for the study were recruited and also reviewed the manuscript.

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