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Molecular Detection and Frequency Rate of *Candida albicans* isolated from Women with suspected Vulvovaginal Candidiasis and attending Omdurman Maternity Hospital (Omdurman, Sudan)

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Abstract

Background: *Candida* vulvovaginitis is common and is considered the third condition of all cases of vulvovaginitis among reproductive-aged women; and *Candida albicans* is the most common pathogen responsible for this pathology. Vulvovaginal candidiasis is a fungal infection caused by overgrowth of *Candida* species affecting the genital tract as opportunistic pathogen.

Objective: To perform molecular detection and to estimate the frequency rate of *Candida albicans* isolated from women with suspected vulvovaginal candidiasis and attending Omdurman Maternity Hospital (Omdurman, Sudan).

Materials and methods: A total of 96 vaginal swabs specimens were collected from both pregnant and non-pregnant women with symptoms of vaginal infection. Specimens were tested by microscopic examination and culture on Sabouraud dextrose agar (SDA). Colonial morphology, wet preparation, Gram′s stain, and germ tube test (GTT) were performed for identification of the isolated organisms.

DNA extraction was performed on the pure fresh culture colonies using the guanidine polymerase chain reaction (PCR) method. A structural questionnaire was conducted to collect demographical and clinical data from all patients investigated.

Results: The results showed *Candida* isolates in 22 out of the 96 vaginal swabs investigated by the colonial morphology, wet preparation, Gram′s stain, and GTT techniques. Positive *C. albicans* (*C.albican*s) isolates were 14 (63.6%) by the GGT method and 17 (77.3%) by the PCR technique. While negative *Candida* albicans specimens were 8 (36.4%) by the GGT method and 5 (22.7%) by the PCR technique.

*C.albican*s showed a significant growth (14/17%) among pregnant women ($p = 0.03$) with the highest frequency rate among nulliparous women, those in the third trimester, and those of more than 7 months pregnancy.

Conclusion: Vulvovaginal candidiasis was more common among nulliparous women, those in the third trimester, and those of more than 7 months pregnancy.

Keywords: *Candida albicans*, Pregnant women, Vulvovaginitis, Maternity hospital, PCR, GTT.

Introduction

Vaginal candidiasis (VC) is one of the most common type of vaginal infections (vaginitis) among women in the fertile period, and also is the most frequent and most important fungal disease of vaginal content. The normal bacterial flora of vaginal mucosa acts as a barrier to the overgrowth of fungal infections due to *C.albicans*. Loss of this normal flora is one of the main predisposing factors to an infection by *C.albicans*[1].
70% of women were reported to have *Candida* vulvovaginitis at some point in their lifetimes. The most common responsible pathogen is *C. albicans* (in about 90% of cases), and most of the remaining cases are caused by non-*C.albicans*. The detailed epidemiological data of *Candida* vulvovaginitis in Sudan is not available. Because of the wide availability of over-the-counter treatment, many patients with *Candida* vulvovaginitis do not attend hospitals for medical care. During pregnancy, the high levels of reproductive hormones provide a greater amount of glycogen in the vagina, thus providing a good source of carbon for *Candida* growth[2].
Candida vulvovaginitis occurs when *Candida* species superficially penetrate the mucosal lining of the vagina and cause an inflammatory response. Patients may present with a discharge, which is typically thick and adherent, or with excoriations, external dysuria, vaginal itching, vaginal burning, dyspareunia, or swelling. The majority of studies isolating yeast from vulvovaginitis patients have shown that the recovery of fluconazole-resistant *C. albicans* isolates to be an unusual event[3]. However, they often have included a small number of isolates. Candida vulvovaginitis is responsible for about one-third of cases, being not a cause of mortality, the morbidity associated with vulvovaginal candidiasis makes it a major cause of mental distress and economic costs; though there are well-recognized limitations of the existing epidemiologic data for vulvovaginal candidiasis. For unknown reasons, *Candida vulvovaginitis* is more prevalent in women in sub-Saharan Africa and other low-income countries than in women in developed countries, affecting up to 55% of women in some studies. In developing countries, there is scanty data on vaginal candidiasis among pregnant women and on the distribution of the vaginal *Candida* species[4].
This study aimed to detect the frequency rate of *C.albicans* responsible for vulvovaginitis among women in Omdurman Maternity Hospital (Omdurman, Sudan).

Materials and methods

This was a descriptive, analytical, cross-sectional study conducted at Omdurman Maternity Hospital (Omdurman, Sudan) during the period from June 2019 to March 2020. The participants were 100 married women aged 17-45 years old, including pregnant and non-pregnant women. The patients presented with either erythema and itching of vulva or vaginal discharge. Verbal consent and structural questionnaire enquiring their age, marital status, duration of symptoms, comorbidities, signs and symptoms of current condition, stage of pregnancy, and history of antibiotic therapy was obtained from each patient. Permission to conduct the study was taken from the Ethical Board of Al Neelain University, and permission to collect the specimens was granted from Omdurman Maternity Hospital (Omdurman, Sudan). All participants were informed of the purpose of the study before collection of the specimens, and verbal consent was

taken from each of them. Exclusion criteria were patients on an antifungal treatment.
High vaginal swabs were collected aseptically from each patient from the vaginal fornix using vaginal wall retractors. The swabs were transferred to the microbiology laboratory and processed immediately. Each swab was streaked on selective Sabouraud dextrose agar (HiMedia, India) plates containing chloramphenicol (0.05g/l), and incubated at 37°C for 48 hours. Phenotypic identification was made using Gram′s stain. Colonies suggestive of *C.albicans* were confirmed by the germ tube test. Direct wet mount microscopy using 10% potassium hydroxide solution to determine the presence of yeast cells in each specimen.
The DNA extraction was performed on pure fresh culture colonies using the guanidine chloride method as described by Gassoum and his colleagues (2014)[5]. The isolated pure growth colonies were washed three times in 5 ml phosphate. Then 2 ml white blood cells lysis buffer and 20 µl proteinase K (10 mg/m) were added, vortexed and incubated at 37°C overnight. Then 1 ml guanidine chloride and 350 ml ammonium acetate were added, the tubes were vortexed and incubated at 65°C in an oven for 2 hours. Then the supernatant was mixed with 2 ml pre-chilled chloroform, vortexed and centrifuged at 6000 rpm for 20 minutes. The supernatant was transferred to a new Falcon tube and the volume was completed to 10 ml with pre-chilled absolute ethanol and incubated overnight at -20 °C. After incubation the tubes were centrifuged at 6000 rpm for 25 minutes. Then the ethanol was poured into disposable bottles, and the same step was repeated with 4 ml 70% ethanol. The tubes were left to air-dry, 130 µl TE buffer was added and incubated at 4°C for completion of DNA elution. Nanodrop ND 1000 spectrophotometer (Nano Drop Technologies, Inc.) was used to determine the quality and quantity of DNA concentration.
PCR amplification was performed in a PCR mixture containing 5 µl per mix (iNtRON, Seongnam, Korea), 22 µl deionized sterile water, and 1µl of each of the forward and reverse primers of *C. albicans* shown below:
* F-TTT ATC AAC TTG TCA CAC CAG A
* R-ATC CCG CCT TAC CAC TAC CG[6]
1 µl of the genomic DNA was used to serve as DNA template to a final volume of 25 µl. The PCR cycling conditions were as follows:
* An initial denaturation step of 5 min. at 95°C followed by 35 cycles of 45 sec at 94°C, 45 sec at 55°C, and 45 sec at 72°C, with a final extension of 5 min at 72°C. The reactions were carried out in an ESCO thermocycler (AERIS- BG096, China).
The PCR products were analyzed using 2% agarose gel stained with ethidium bromide (10 ng/100 ml) and visualized under a UV transilluminater device.
Analysis of data was performed by means of the Statistical Package for Social Sciences (SPSS) software program, version 22. Correlation of personal and socio-demographic data was analyzed and presented in the form of tables and figures. Significance of differences was determined using chi-square test and statistical significance was set at p value < 0.05.

Results

Culture of 96 high vaginal swabs showed 22 (22.9%) positive *Candida* species. By the GTT method, the frequency rate of positive *C.albicans* was 14 (63.6%), while by the PCR technique,

the frequency rate of positive *C.albicans* was 17 (77.3%).
As shown in Table (1), the positive GTT *Candida albicans* cases were found significantly correlated with those positive with PCR techniques (p = 0.000).

Table (1): Correlation of *Candida albicans* cases detected by GTT and PCR techniques

	Candida albicans			
	GTT Positive	GTT Negative	Total	p. value
PCR				0.000
Positive	12 (54.5%)	5 (22.7%)	17 (77.3%)	
Negative	2 (9%)	3 (13.6%)	5 (22.7%)	
Total	14 (63.6%)	8 (36.4%)	22 (100%)	

On the other hand, the frequency rate of positive *C.albican*s among pregnant women was (82/17%) as compared to (0/0.0 %) among non-pregnant women. This difference was statistically insignificant (p = 0.198).
As shown in Table (2), the correlation of *Candida* species growth with gestation among pregnant women was found statistically significant (p = 0.03). The frequency rate of positive *C.albicans* was highest among pregnant women in the third trimester (11/28.2%), and those of more than 7 months pregnancy (3/10%).

Table (2): Correlation of *C.albican*s detected among the pregnant women

Gestation	Positive *C. albicans*	Negative *C. albicans*	No growth	Total	p - value
First trimester	0 (0.0%)	0 (0.0%)	14 (100%)	14 (100%)	0.03
Second trimester	3 (10%)	2 (6.6%)	25 (83.4%)	30 (100%)	
Third trimester	11 (28.2%)	4 (10.2%)	24 (61.6%)	39 (100%)	
Not pregnant	0 (0.0%)	2 (15.4%)	11 (84.6%)	13 (100%)	

The frequency rate of positive *C.albicans* was (2/40 %) among nulliparous pregnant women, (1/9.1%) among para one pregnant women, and (11/13.8٪) among multiparous pregnant women. Whereas the frequency rate of negative *C.albicans* was (0/0.0 %)

among nulliparous pregnant women. This difference was statistically insignificant (p = 0.07).

Table (3): Frequency rates of positive *C. albicans* according to age incidence, symptoms of itching and vaginal discharge, past history of vulvovaginal candidiasis, and antibiotic therapy

Parameter	Positive *C. albicans*	Negative *C. albicans*	p - value
Age incidence			
17-27 years	6 (14.3%)	7 (16.7%)	0.104
28-38 years	8 (16%)	1 (2%)	
39-49 years	0 (0.0%)	0 (0.0%)	
Itching			
Present	7 (20%)	2 (5.7%)	0.44
Absent	7 (11.5%)	6 (9.8%)	
Vaginal discharge			
Present	14 (14.7%)	8 (8.4%)	0.86
Absent	0 (0.0%)	0 (0.0%)	
Itching + Vaginal discharge			
Present	7 (20%)	2 (5.7%)	0.86
Absent	7 (11.5%)	6 (9.8%)	
Past history of vulvovaginal candidiasis			
Present	9 (15.5%)	3 (5.2%)	0.37
Absent	5 (13.2%)	5 (13.2%)	
Antibiotic therapy			
Present	2 (28.5%)	2 (28.5%)	0.05
Absent	12 (13.5%)	6(6.8%)	

The frequency rate of *C.albicans* according to duration of symptoms of vulvovaginal candidiasis was (4/6.5%), (5/23.8%), (2/33.3%), and (3/42.8%) among pregnant women with duration of less than month, with duration of 1-3 month, with duration of 4-7 months, and with duration of more than 7 months respectively. This difference was statistically significant (p = 0.05).

Table (3) shows the frequency rates of positive *C.albicans* according to age incidence, symptoms of itching and vaginal discharge, and past history of vulvovaginal candidiasis. These differences were statistically insignificant (p = 0.104; p = 0.44; p = 0.86; p = 0.37 respectively). However, the frequency rate of positive *C.albicans* according to antibiotic therapy was statistically significant (p = 0.05).

Discussion

Vulvovaginal candidiasis (VVC) is the second most frequent infection of the female genital tract, worldwide, and it is suspected on irritation of vulva, vagina, or both. Numerous studies had elucidated that *Candida albicans* is responsible for the greatest number of symptoms associated with vaginal candidiasis[6].

In the present study, the number of positive *Candida* cultures were (22/22.9%), a finding similar to that reported by Nyirjesy and his co-authors (2005)[7] who investigated a number of high vaginal swabs. Also, Al Tayyar and his colleagues (2017)[8] reported that the prevalence rate of vaginal candidiasis was (24%) among the pregnant and non pregnant women studied.

In this study, 63.6% of *Candida* isolates were identified as *C. albicans* using GTT, whereas 77.3% were identified using PCR. Similar, findings were reported by Khan and his co-workers (2007)[9] who reported that 53% of *C. albicans* species were detected by GTT method. Furthermore, in this study positive PCR and positive GTT was shown among *C. albican*s with a frequency rate of 12 (54.5%) out of 22 cases; and there was 5 cases with positive PCR but negative GTT (22.7%).

Molecular methods can give definitive identification with one-day results and do not need previous cultures. Thus, diagnosis can be made directly from the specimen collected from patients, providing valuable information for patient management. Hence the PCR technique is more specific than GTT in the diagnosis of *C. albicans* .

In the present context, is was found that the prevalence rate of vaginal candidiasis was (17%) among pregnant women and (7%) among non-pregnant women. This may due to hormonal factors arising during pregnancy that can play a role in enhancing *Candida* colonization and serving as a risk factor causing vagina infection. These findings were similar to those reported by Feyi and Amadi (2006)[7]. Similar findings were reported from Kenya by Menza and his co-authors (2013)[10] where high prevalence rate of vaginal candidiasis was detected among pregnant women.

On the other hand in this study, most of *C.albicans* growth was found among pregnant women in the third trimester and among nulliparous women. Such finding was found similar to that reported by the study conducted at Michigan University (USA) which showed that most of *C.albicans* growth was detected in nulliparous and pregnant women in the 3rd trimester[11].

The frequency rate of *C.albicans* according to the duration of symptoms of vulvovaginal

candidiasis reported in the present context was found more prevalent (42.8%) among pregnant women with the duration of more than 7 months pregnancy. This finding was similar to that reported by Eckert and his colleagues[12.]

The highest prevalence rate of vaginal infection in this study was noted among patients in the age range 17-27 years, followed by those in the age range 28–38 years. This finding was consistent with the report of Al Tayyar and his co-workers (2016)[8] who reported that the highest prevalence rate of vaginal infection (44.4%) was noted in the age range 16-25 years, followed by those (34.9%) in the age range 26-35 years. Also, in the present study, all *Candida* species isolated were found among pregnant women with vaginal discharge and *Candida albicans* was predominant (Table 3). This finding agreed with that reported by the study of Zhou and his co-authors (2009)[13] who found that vaginal discharge was the most common symptom among pregnant women, followed by itching.

Furthermore in this current study, candidiasis was found most more common among women under antibiotic therapy as compared with those not under antibiotics therapy (57% vs 20.3%). This finding was similar to the finding of Ehan (2017)[14] who found a significant association between vaginal candidiasis and the use of broad spectrum antibiotics.

Recommendation: The high frequency rate of *C.albicans* associated with vulvovaginal candidiasis may substantiate the importance of periodic monitoring of pregnant women for early detection, accurate diagnosis, and proper therapy to prevent adverse outcomes during pregnancy and child birth, since this infection can be asymptomatic.

Conclusion: Vulvovaginal candidiasis was more common among nulliparous women, those in the third trimester, and those of more than 7 months pregnancy.

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