Candidiasis among single and married women attending Murtala Muhammad Specialist Hospital, Kano-Nigeria

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Publication history: Received on 24 May 2020; revised on 30 May 2020; accepted on 02 June 2020

Article DOI: <https://doi.org/10.30574/wjbphs.2020.2.3.0031>

Abstract

Candida species are among important opportunistic pathogens causing candidiasis in human worldwide. These yeast species are of public health concern nowadays. The study was aimed at evaluating the prevalence of candidiasis among single and married women. Clean catch midstream urine were collected from the enrolled subjects, the samples were inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 48h. Gram's staining technique, germ tube test and KOH test were the techniques performed on purity plate before subculture on to Chromogenic agar for phenotypic speciation. Out of the 204 samples analysed, a prevalence of 75% was obtained. Four different *Candida* species were isolated; *Candida albicans* 52(69.33%), *Candida galabrata* 8(10.8%), *Candida krusei* 11(14.6%), and *Candida tropicalis* 4(5.33%). Based on the results obtained, married women were more prone to the disease 49(65.3%) with singles having 26(34.7%). According to age groups, 21-30 had highest infection rate 34(45.3%) compared to other groups. Out of the four *Candida* species, *C. albicans* were more isolated 52(69.3%) while *C. tropicalis* had the least 4(5.33%). It is therefore important to give opportunistic pathogens like *Candida* species attention in women especially among married ones.

Keywords:Candidiasis; Single and Married women; Candida species

1. Introduction

Candidiasis is a disease condition coursed by yeast of the genus *Candida*, which has several species that are opportunistic in nature and affects mostly immunocompromised individuals. In women, the most prominent condition they faced due to these species is the Vulvovaginal candidiasis (VVC) and oral candidiasis.

As a disease, vulvovaginal candidiasis, is not regarded as sexually transmitted, because it do occur also in celibate women (women that do not marry and do not engage in sexual activities due religious believes) and children, however, this does not mean that *Candida* cannot be transmitted sexually [1,2]. Some studies indicated that approximately two-thirds of women experience at least an episode of vulvovaginal candidiasis in their lifetime, and up to 50% of them may have multiple episodes of the disease [3,4]. From available literature, studies were done on pregnant women, immunocompromised individuals, especially diabetics subjects, individuals on broad-spectrum antibiotic therapy, women on oral contraception with high estrogen content, and HIV-positive subjects, while few studies were on otherwise immunocompetent women whether single or married [5].

For VVC in women that are either single or married, clinical manifestations includes pruritus, irritation, cottage cheese-like vaginal discharge and burning sensation [6]. Among married women, many studies have indicated high prevalence of *Candida* among pregnant women than that of nonpregnant ones, and believed to increase with the progression of the pregnancy period [78]. According to some established data, candidiasis during pregnancy might be associated with increased risk of complications, such as preterm labor, chorioamnionitis, and congenital cutaneous candidiasis among

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other conditions [9]. In candidiasis among women, many factors including physiologic changes, such as elevated hormone levels, decreased cellular immunity, reduced vaginal pH, and increased vaginal glycogen concentration, are considered as major attributes associated with higher risk of vaginal candidiasis are believed to play key roles [10,7,11].

As a results of broad-spectrum antibiotic usage, *Candida* colonization frequently do occurs and has been linked to a decreased colonization of *Lactobacillus*, possibly due to the interference with epithelial binding sites in the body [12]. In a situation, when balance between normal bacterial flora, *Candida* and immune defense mechanisms is disturbed, then, colonization changes to infection, in which according to records, *C. albicans* accounts for the highest rate, accounting some times for up to 85-90% of candidiasis episodes [12].

Other candida species (the non-albicans *Candida* species), such as *C. glabrata*, *C. tropicalis*, and *C. krusei* are now emerging as serious causes of candidiasis and have considerable variations with regards to virulence, epidemiology, and susceptibility profile to antifungal agents [6].

2. Material and methods

2.1. Study Area

The research was carried out at Aminu Kano Teaching Hospital (AKTH), Kano, situated in Kano metropolis and a referral center for both private and public health institutions in and the around neighboring states. Kano lies between latitude 11°30'N and longitude 8°30'E. Kano state borders Katsina to the north-west, Jigawa state to the north-east, Bauchi state to the south-east and Kaduna state to the south-west. The total land area of Kano state is 20,760 square kilometers with a population of 9,383,682 based on the official 2006 National Population and Housing Census [13].

2.2. Study Design

The research was cross-sectional prospective study

2.3. Study Population

The study population, were single and married women attending Murtala Muhammad Hospital Kano.

2.4. Inclusion criteria

Married and single subjects that are suspected of having candidiasis and not on any antifungal medication, attending Murtala Muhammad Specialist Hospital, Kano.

2.5. Exclusion criteria

Those women willing to participate in the research, but on some antifungal drugs were excluded from the study.

2.6. Sample size determination

The sample size was obtained using the formula stated by [14], and calculated using prevalence of (14%) from study conducted by [15].

Where

n = number of samples

z = statistic for level of confidence at 95%=1.96

p = prevalence =14%

d = allowable errors of 5%, (0.05)

=185

with attrition of 10%, =204

2.7. Ethical consideration

Ethical approval to conduct the research was obtained from the research ethics committee of Kano state Ministry of Health. The participant (subject) consent was also sought for prior to the administration of the questionnaires.

2.8. Sample collection and processing

Clean catch midstream urine was collected from each patient in sterile universal container, each container was labeled before issued to the participants.

The collected clean catch midstream urine samples were processed immediately. In case of any delay, the samples were stored at 2-8oC in the fridge.

2.9. Macroscopy

The color and presence of blood and turbidity of each sample were macroscopically examined and recorded accordingly.

2.10. Microscopy

2.10.1. Direct Gram's staining technique

After inoculating the samples on culture media, smears were prepared by placing a drop of the urine sample on a clean glass slide, spread, air dried, and heat fixed by quickly passage over flame three (3) times. The primary stain (crystal violet) was applied for 1 minute and rinsed with water then the smear was flooded with Gram's iodine for a minute and rinsed with water, thereafter, it was decolorized with acetone briefly and rinsed with water, the smear was finally counter stained with neutral red for 1 minute, was rinsed with water, air dried and examine microscopically using oil immersion objective [16].

2.10.2. Wet preparation (KOH mount)

A drop of each sample was transferred onto clean grease-free slide and 10% KOH was dropped onto the sample and covered with a cover slip. The slide was heated gently over flame, and then allowed to stand for 5 minutes. Finally, the slide was examined microscopically under 10x and 40x objective lens [17].

2.11. Inoculation onto culture media

The urine samples were inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 24 to 48 hours for growth of colonies.

2.12. Purity plate

Colonies obtained from the primary plates were subcultured onto Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 24 hours to obtained pure isolates.

2.13. Gram's staining technique

All colonies obtained from the purity plates were Gram stained to confirm large budding oval yeast before further tests were continued.

2.14. Germ tube test

About 0.5ml of serum was transferred into small test tube, the colonies from pure culture was picked and gently emulsified in the serum and incubated at 37°C for 2-3 hours. After the incubation period, a drop of the preparation was transferred to a clean grease-free glass slide and covered with cover slip and examined using 40× objective lens at thirty minutes interval [17].

2.15. Subculture on Chromogenic Agar for phenotypic Speciation

The isolates obtained from purity plates were subcultured onto chromogenic agar and incubated at 37°C for 24-48 hours, after the incubation period, the colonies appeared with different colours depending on the species types.

2.16. Statistical Analysis

The data collected and results obtained were analyzed using SPSS software version 20 and presented in tables.

3. Results and discussion

During the research a total of 204 subjects were enrolled, out of which a prevalence of 75(36.7%) was obtained, table 1. In the course of the study four *Candida* species were isolated with the following respective frequencies 52, 8, 11 and

4, for *Candida albicans*, *Candida glabrata*, *Candida kusei* and *Candida tropicalis* respectively. This shows that *Candida albicans* was the predominantly isolated *Candida* specie during the study, table 2. Distribution of the subjects based on marital status was equal, in which one hundred and two participants were enrolled in each group (married and single), making a total of two hundred and four subjects, table 3. The distribution of the *candida* species based on the marital status showed that, *candida albican* had the highest isolation rate in both married and the single participants with 33 and 19 isolation rates respectively, *Candida krusei*, *C. glabrata* and *C. tropicalis* had 9 and 2, 6 and 2, and 1 and 3 isolate for the married and single subjects respectively, table 4. With respect to age groups, age bracket of 21-30 had the highest infection rate in the two categories with 34 isolates representing 45.3%, followed by age limit of 31-40 with 18 isolates representing 24% while the least affected group was 61-70 which had only 1 isolate representing 1.3%, table 5.

Table 1 The prevalence of candidiasis observed

Status of the subject	Frequency	Prevalence (%)
Positive	75	(36.7)
Negative	129	(63.2)
Total	204	(100)

Table 2 The *Candida* species isolated

Candida Species	Frequency	Prevalence (%)
<i>C. albican</i>	52	(69.3)
<i>C. glabrrata</i>	8	(10.8)
<i>C. krusie</i>	11	(14.6)
<i>C. tropicalis</i>	4	(5.33)
TOTAL	75	(100)

Table 3 Frequency of isolates among the study groups

Status of the subject	Frequency	Percentage (%)
Single	102	50
Married	102	50
Total	204	100

Table 4 The species distribution based on marital status

Candida species	Married Number (%)	Single Number (%)	Total Number (%)
<i>C. albican</i>	33(67.4)	19(73.1)	52(69.3)
<i>C. krusie</i>	9(18.4)	2(7.7)	11(10.8)
<i>C. glabrata</i>	6(12.2)	2(7.7)	8(14.6)
<i>C. tropicali</i>	1(2.0)	3(11.5)	4(5.33)
Total	49 (100)	26 (100)	75(100)

Table 5 Age wise distribution of the Candida species among the study groups

Age group	Number Examined	Married Positive Number (%)	Single Positive Number (%)	Total (%)	P value
10 – 20	29	8 (16.4)	3 (11.6)	11 (14.7)	0.891
21 – 30	102	21 (42.9)	13 (50.0)	34 (45.3)	
31 – 40	46	11 (22.4)	7 (26.9)	18 (24.0)	
41 – 50	13	5 (10.2)	2 (7.7)	7 (9.3)	
51 – 60	12	3 (6.1)	1 (3.8)	4 (5.4)	
61 – 70	2	1 (2.0)	0 (0.0)	1 (1.3)	
Total	204	49 (100)	26 (100)	75 (100)	

4. Discussion

The overall prevalence obtained in this study was 75(36.7%). This in close agreement with Enweani, 2015, in a study in Jos University Teaching Hospital, Jos, Plateau state, where they recorded a prevalence of 40.78% of the total samples examined.

Based on the species isolated, the study showed that *Candida albicans* had the highest isolation rate of 52(69.3%) followed by *Candida krusei* 11(14.6%), and *Candida glabrata* 8(10.8%) while *Candida tropicalis* were the least isolated 4(5.3%). However, [18], were able to isolate more species than found in this research with, *C. albicans*, *C. glabrata*, *C. guilliermondi*, *C. parapsilosis*, *C. pseudotropicalis*, *C. stellatoidea*, *C. tropicalis* and *C. viswananthii*. Even in their study, *C. albicans* was also the predominant isolate observed 38.10% followed by *C. tropicalis* 21.43% while the least percentage occurrence was recorded for *C. viswananthii* which had 2.38% [18].

In the research equal chances were given to both the single and the married women enrolled in the study 102 each making a total of 204 participants, this was however different from a similar work [19], in which they used more of the married subjects 287 than the single subjects 155 in their study. Isolation with respect to status, in this study show that, married women had the highest infection rate with 46 isolates while the single had 26 isolates only. However, [20], had extra group apart from married and single to include divorce/widowed in their research and their isolation frequencies were 97.4%, 0.0% and 2.6% respectively.

With respect to age groups of the subjects, age range of 21-30 had the highest infection rate 34 (45.3%) followed by 31-40 with 18(24%) while the age limit of 61-70 had the least frequency of 1 isolate representing (1.3%). This differs from findings of [21], who had different age classification 15-25, 26-35, 36-45 and ≥ 46 with 24, 56, 12 and 2 as their respective isolation frequencies, which indicated that the age range of 26-35 had the highest infection rate 56 which is close to our age range 21-30, that had the highest isolation frequency of 34. They also got the least isolation rate of 2 in their upper limit ≥ 46 which is also close to our findings of 1 isolate in the upper limit of 61-70 ages limit.

5. Conclusion

We can conclude based on the results therefore that, candida infection is one of the established opportunistic mycotic diseases that affects women, and its agents includes both albicans and non albicans candida species, particularly in the study area. It also showed that married women are more prone than those that are single.

Compliance with ethical standards

Acknowledgments

Our acknowledgement goes to the management of Murtala Muhammad Specialist Hospital, Kano and staff of microbiology department in particular. We also want to acknowledge all those who contributed in one way or the other from the beginning to the end of the research.

Disclosure of conflict of interest

We also wish to state that there is no conflict of interest in whichever among the authors.

Statement of informed consent

Informed consent of all the participants was sought for, before enrolled in the study.

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How to cite this article

Sule H, Kumurya AS and Muhammad BK. (2020). Candidiasis among single and married women attending Murtala Muhammad Specialist Hospital, Kano-Nigeria. World Journal of Biology Pharmacy and Health Sciences, 2(3), 01-07.