(RESEARCH ARTICLE)

Candidiasis among diabetic patients attending Aminu Kano teaching hospital, Kano-Nigeria

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Abstract

Candidiasis is a yeast infection caused by the species of the genus candida that lives as commensal in/on some parts of the body, like gastrointestinal tract, respiratory tract, vagina and even on the skin. However, they later change to opportunistic pathogens under certain conditions especially in suger environment like in diabetic patients, because they are suger loving organisms. The study was to identify the extent of Candida infections among diabetic patients. During the study,Clean catch midstream urine samples were collected from the participants, the samples were inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 37ºC 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 of the isolates. A prevalence of 16.2% was observed with four different candida species (*Candida albicans* 45.7%, *Candida krusei* 28.6%, *candida glabrata* 20% *and Candida tropicalis* 5.7%) isolated. Infection rate according to gender, the result showed that female were the most affected than males and age limit of 31-45 was the most infected group based on the study followed by 46-60 age range while the age range of 16-30 had the least infection rate in the study.

Keywords: Candidiasis; Diabetic patients; *Candida species*; Opportunistic Pathogens

1. Introduction

Historically, yeast of the genus Candida are ubiquitous in nature with opportunistic tendencies, and are part of the normal microbiota of the oral mucosa (OM), gastrointestinal tract and the vagina [1] Fungal colonization and subsequent infection depends on certain factors like the yeast characteristics, in particular, its adhesion ability to epithelial cells, as well as the host's systemic condition like, immunosuppression due to different cause and diabetes among others [2] According to records, *Candida albicans* remain the most abundant species in colonisation of body parts like the oral mucosa in immunocompromised and even healthy individuals; however, other species*: C. tropicalis*, *C. glabrata, C. parapsilosis, C. dubliniensis C. guilliermondii and C. krusei* are also gaining increased prevalence [1]

Candidiasis refers to the disease condition associated with the various species of the yeast genus, Candida which is universally agreed to be a good sugar loving organism, as such find it comfortable to thrive and be associated with diabetic patients.

In itself, diabetes mellitus is a chronic insulin related disorder in which blood suger levels are not properly regulated and that make the body prone to other disease conditions like candidiasis [3]. This category of patients, are at increased risk of vulvovaginal candidiasis [4]. which is the second most frequent infection associated with female genital tract [5]. The species of the genus candida, are opportunistic organism and usually not pathogenic in nature except in an immunocompromised host, where they cause diseases, ranging from superficial form, involving the outer layer of the stratum corneum of the skin to the disseminated infection which has to do with the lungs, vagina and other body parts [6].

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Due to the nature of their disease, diabetic patients usually experienced resistance and recurrent infections of various kinds apart from slow healing process in case of wound or injuries sustained; It is normally considered that, the condition is due, in part to, malfunctioning leucocytes, especially in the presence of uncontrolled blood glucose levels in the affected patients, among the diseases, fungal vaginitis caused by *Candida* species, is more disturbing especially in severely hyperglycemic patients[7]. For that, most gynecologists begin antifungal therapy at the first patient's visit, even without a culture of vaginal discharge [8].Of cause, this is improper diagnosis and wrong treatment direction.Example, in one study, up to 28% *Candida* species were isolated from vaginal discharge obtained during initial visit of women with vaginitis;which will have been missed if laboratory diagnosis was not south for,it is therefore believed that causes of recurrent disease or resistance to therapy were due to false diagnosis of the causative organism leading to unsuitable treatment [9]. But on the other hand, even with correct diagnosis, background factors, especially those related to systemic diseases like diabetes mellitus, caused treatment failure [10].

2. Material andmethods

2.1. Study area

The study was carried out at Aminu Kano Teaching Hospital (AKTH), Kano, situated in Kano metropolis. Kano lies between latitude 11º 30`N and longitude 8º30`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 [11].

2.2. Study design

The research was a cross-sectional prospective study

2.3. Study population

The study population was diabetic patients attending diabetics' clinic of Aminu Kano Teaching Hospital (AKTH), Kano Nigeria

2.4. Inclusion criteria

Diabetic patients attending clinic (diabetics clinic) of Aminu Kano Teaching Hospital, that agrees to take part and not on any antifungal medication.

2.5. Exclusion criteria

Non-diabetic patients attending Aminu Kano Teaching Hospital, diabetic patient that did not give their consent and diabetic patients on antifungal medications.

2.6. Sample size determination

The sample size of this study was determined using the formula as follows:

$n = (z^2 p(1-p))/d^2$

Where:

n = number of samples

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

p = prevalence of candidiasis among diabetic patients = 18.8% [12].

d = allowable error of 5%, (0.05)

$n = (〚1.96〛^2 \times 0.188(1-0.188))/〚0.05〛^2 = 213$

The total sample size was calculated to be 213

2.7. Ethical approval

Ethical approval to conduct the research was obtained from the Research Ethics committees of AKTH. The participants (subjects) consents were sought prior to the administration of the questionnaires.

2.8. Sample collection and processing

Early morning midstream urine samples were collected in labeled sterile universal containers and processed immediately, when delay was anticipated, the samples were stored in fridge at 2-8 °C.

2.8.1. Macroscopy

The appearance of the urine samples like colour, turbidity and blood tinge were applicable were checked and recorded.

2.8.2. Microscopy

Direct Gram's staining technique

Smear was prepared by placing a drop of the centrifuged urine deposit onto a clean glass slide, then emulsified and air dried, the smear was then heat fixed by quickly passing over flame three (3) times. The primary stain (crystal violet) was applied for 1 minute and rinsed with water then Gram's iodine was applied and rinsed again, the smear was decolorized with acetone briefly and rinsed with water, it was then counterstained with neutral red for 1 minute, rinsed with water and allowed to air dried and examined microscopically using oil immersion objective lens [13].

Potassium hydroxide (KOH) wet mount preparation

The collected samples were dropped on clean grease-free slides containing a drop of 10% KOH, then, covered with a cover slip. The smear was passed quickly over a flame two to three times and then allowed to stand for 5 minutes. Finally, the slide was examined microscopically [13]

2.9. Inoculation onto culture media

Samples were inoculated onto Sabouraud's dextrose agar (SDA) suplimented with chloramphenicol and incubated at 37 °C for 48 h [13]

2.9.1. Purity plate

Isolates obtained from the primary culture plates were sub-cultured onto SDA for 24 hours at 37 °C to obtain pure colonies [14].

2.9.2. Gram staining technique

Smear was prepared by placing few colonies from pure culture onto a clean glass slide, then emulsified and air dried, the smears were then heat fixed by quickly passing over flame three (3) times. The primary stain (crystal violet) was applied for 1 minute and rinsed with water then Gram's iodine was applied and rinsed again, the smear was decolorized with acetone briefly and rinsed with water, It was then counterstained with neutral red for 1 minute, rinsed with water and allowed to air dried and examined microscopically using oil immersion objective lens [13]

2.9.3. Germ tube test

Few colonies from the pure cultures were suspended in 0.5 ml of serum in test tubes, all the tubes were incubated at 37 °C for 3 hours and a drop of the preparation was placed on a glass slide and covered with cover slip and examined for the presence or absence of germ tubes [14]

2.9.4. Culture onto chromogenic candida agar for phenotypic speciation

Isolates from purity plates were inoculated onto CHROM candida agar, using sterile inoculating loop and incubated at 37 °C for 48 hours. This method is based on the differential colony colours appearance depending on the Candida specie [15] Identification of the yeast was based on their colony colour and morphology [16]. 2.11 Statistical analysis

The data collected and results obtained were analyzed using statistical package for social sciences SPSS software package version 20.0 and presented in tables.

3. Results

In the study, two hundred and sixteen (216) subjects were recruited, in which a total prevalence of 35 (16.2%) was obtained and *Candida albican* found to be the dominant isolate in the study area. According to the research, four *candida* species were isolated as follows; *Candida albicans* 16(45.7%), *Candida krusei* 10(28.6%), *candida glabrata* 7(20%) and *Candida tropicalis* 2(5.7%), Table 1. Genderwise distribution of the isolates showed that, females were the most infected, with males having 3 *Candida albicans*, 4 *Candida krusei* and 1*Candidaglabrata* while the females had 13 *Candida albican,* 6*Candida krusei*, 10(90%) *Candida glabrata* and 2 *Candida tropicalis,* table 2. Based on age groups of the participants, age range of 31-45 were the most at risk group with the frequency of isolation of 4, 5 and 4 for *Candida albican*, *Candida krusei* and *Candida glabrata* isolates respectively. While the age limit of 16-30 had the least; with only 2 *Candida albicans* and 2 *Candida krusei* and 1 *Candida tropicalis.* Other age groups have were 46-60 with 6 *C.albicans,* 2 *C. krusei* 1 *C. galbrata and* 1 *C. tropicalis*while > 60 age limit had 4 *C. albicans,* 1 *C. krusie* and 1 *C. galbrata* during the study, table 3

Table 1 The Candida species isolated

Candidsa species	Fequency	Percentage
Candida albican	16	45.7
Candida krusei	10	28.6
Candida glabrata	7	20
Candida tropicalis	2	5.7
Total	35	100

Table 2 Genderdistribution of the isolates

Gender	C. albicans	C. krusei	C. glabrata	C. tropicalis	P-value
Male	3(18.8%)	4(14.3%)	1(10%)	0(0.0%)	0.00
Female	13(81.2%)	6(85.7%)	10(90%)	2(100%)	
Total	16(100%)	10(100%)	7()100%	2(100%)	

Table 3 Age group distribution of the Candida species isolated

Age group	C. albicans	C. krusei	C. glabrata	c. tropicalis	P-value
16-30	2(12.5%)	2(20.0%)	0(0.0%)	1(50%)	0.623
31-45	4(25.0%)	5(50.0%)	4(57.1%)	0(0%)	
46-60	6(37.5%)	2(20.0%)	1(14.3%)	1(50%)	
>60	4(25.0%)	1(10.0%)	2(28.6%)	0(0%)	
Total	16(100%)	10(100%)	7(100%)	2(100%)	

4. Discussion

Candidiasis as an opportunistic disease affects immunocompromised and people with underline conditions like diabetic patients. In this study, four different *Candida* species were identified which include;*Candida albicans, Candida krusei, Candida glabrata and Candiida tropicalis* with 16(45.7), 10(28.7), 7(20.0), 2(5.7) as their isolation frequencies respectively. [17] report up to five different *Candida* specie which includes *Candida albicans, Candida glabrata, Candida tropicalis, Candida kefyr* and *Candida famata* with 22(39.3), 7(12.5), 6(10.7), 1(1.8) and 1(1.8) as their isolation frequencies respectively. However, in both the situations, *Candida albicans* had predominated with highest isolation frequencies, although two additional different isolate were obtained in their study (*Candida kefyr* and *Candida famata)* which could be attributable to locality differences. [18], also reported four *Candida* species as obtained in our study, but with *Candida krusei* been replaced by *Candida paapsilosis,* the speciesand their isolation frequencies were *Candida albicans* 64, *Candida glabrata* 11, *Candida tropicalis* 9 and *Candida parapsislosis* 9 as found in their research.

In another study, [19], were able to isolate*Candida albican* with highest isolation frequency (20 isolates) against the non albicans species, while then*on albicans* species had 17, 2 and 1 for *Candida glabrata, Candida fatmata* and *Candida tropicalis* respectively. On the other hand,[20], isolated only three species *Candida albicans, Candida duliniensis* and *Candida troppicalis* but still *Candida albican* had the greater portion of the isolates 29(80%) while16(17%) and 1(3%) were for the *Candida dubliniensis* and *Candida tropicalis* respectively.

Distribution of the isolates based on gender showed that Females were at higher risk than males with isolation rates of 31 and 8 respectively. [21], reported however, that males were slightly more infected than the females with 50% and 46.7% isolation rates respectively. However, [22], found that, females suffers the most when compared to the males subjects with 44.3% for males and for females 55.7% subjects respectively. With regards to age limits, the age bracket of 31-45 had the highest infection rate 13, followed by 46-60 with10 while the least was 16-30 with only 5 isolates identified. [20], had the highest isolation rate in the age range of 60-65 with 19% followed by 25-30 and 45-55 which had 10 isolates each while age range of <15 had 0%. [21], used only two age categorization, that is, those subjects with age greater than fifty (>50) in one group and those with age lessthan fifty (< 50) in the other group. Base on that, they found the age range of >50 at higher risk with 56.2 while those of < 50 had 33.8 as their isolation frequency.

5. Conclusion

The rate of infection with candida species observed in this study further highlight the risk of diabetic patients to other opportunistic diseases and female were shown to be more prone to candidiasis in the study area. The way forward is people to always check their suger levels even if they were not diabetic to reduce the risk of this infection.

Compliance with ethical standards

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Disclosure of conflict of interest

We want state that, there is no conflict of interest in whichever among the authors.

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