



ORAL CANDIDIASIS AMONG HIV PATIENTS ATTENDING AMINU KANO TEACHING HOSPITAL

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ABSTRACT

Candidiasis as an opportunistic infection that comes up in many different forms, depending on the part of the body involved and the category of patients affected. Oral candidiasis affects the oral cavity, and is a common phenomenon among HIV patients who are subject to immune depression. This study was to determine the burden of oral candidiasis among HIV patients in the study area. Samples were collected using sterile cotton tipped swabs and processed immediately. In the process, direct gram staining technique and KOH mount was carried out prior to culturing on Sabouraud dextrose agar followed by sub-culturing on chromogenic agar, to help in the phenotypic speciation of the different candida isolates after germ tube test was carried out. A total of 109 *Candida* species were isolated in this study, that include *Candida albicans* 72(66.1%), *Candida glabrata* 13(11.9%) and *Candida krusei* 24(22.0%). It was also discovered that age limit of 21-30 had the highest infection rate 32(29.4%) while the age range of 1-10 had the least 2(1.8%). Gender wise distribution indicated that 68(62.4%) of the 109 positive cultures were from females while 41(37.6%) were of males origin. In conclusion, results have revealed how *Candida* species are in close association with HIV infected individual which is of course a course for public health concern

Keywords: Oral Candidiasis, HIV patients, Opportunistic infections, Candida species

INTRODUCTION

Many characteristics determines the differentiation of yeast species, which exist as unicellular organism and reproduce by budding. They differ from one another on the basis of their sizes and shapes, the formation of pseudohyphae or true hyphae, the mechanism of daughter cells formation, presence or absence of capsules, and presence or lack of sexual spores, in addition to physiologic data obtained from biochemical tests. According to data obtained from National Nosocomial Infections Surveillance System conducted in the United States, it revealed a 487percentincrease in candida blood stream infections between 1980 and 1989 (Banerjee *et al.*, 1991), mucosal and disseminated candidiasis are the two major forms of candida infections normally observed.

As it normally happen to other opportunistic infections worldwide, the introduction of highly active antiretroviral therapy (HAART) has led to a decreased incidence of oral and oesophageal candidiasis (Mocroft *et al.*, 2005). Normally, in HIV/AIDS patients, it is a common presentation to have mucosal candidiasis, in the form of vaginal, oesophageal or oral infections. According to reported data, incidence of oral candidiasis in HIV infected patients varies from 7% to 93% in the infected individuals, depending on certain factors like; the nature of the patient, diagnostic criteria, and study methods used, this was proved by study, on local HIV-infected women

which revealed prevalence of oral candidiasis of 9% while that of vaginal candidiasis was 28, the infection sometimes tend to persist in these group of patients and are associated with higher HIV viral load and a lower CD4count (Duerr *et al.*, 2003).

According to Centre for Disease Control (CDC) revised case definition 1993, vaginal candidiasis which is frequently, persistently, or poorly responsive to therapy, as well as oral candidiasis, became a designated HIV-associated category B condition, according to records, however, the data on recurrent vaginal candidiasis as a sentinel of HIV infection was questioned, as it has not been supported by prospective controlled studies. It has also been classified that oesophageal candidiasis is an AIDS-defining condition, occurring with a CD4<200/ μ L and usually in conjunction with oral candidiasis.

In a study, by Akpan and Morgan. (2002), oral candidiasis (OC) falls into common opportunistic infections in immunocompromised individuals, as it was found to occur in up to 95% of immunodeficiency virus (HIV)-infected individuals during the course of their illness (Feigal *et al.*, 1991; Dupont *et al.*, 1992 and proved to be a prognostic indicator for acquired immune deficiency syndrome (AIDS) (Meurman *et al.*, 2007; Thanyasrisung *et al.*, 2014) . An increased prevalence of severely immunocompromised conditions, has been observed in some sub-Saharan African countries, which is believed to be

associated with higher incidence of opportunistic infections in the region (Hodgson *et al.*, 2002).

As widely documented, OC is mainly due to *Candida albicans*, it has also been estimated that 70% of HIV positive people in sub-Saharan Africa, are at risk of the disease (Guida *et al.*, 1998), it is also known to accounts for up to 81% of cases among HIV patients (Sangeorzan *et al.*, 1994). Other reports also suggest that, between 17% and 75% of healthy living people are colonized by *Candida* species (Bastiaan *et al.*, 1982; Mushi *et al.*, 2016). However, 20%-40% of immunocompromised people comes down with the disease as a result non-*albicans Candida* species colonization of their oral cavity sometimes in life (Kuhn, 2004; Li L *et al.*, 2007; Mushi *et al.*, 2016). It was noted also that, rise in prevalence of oral candidiasis among African HIV infected patients ranges from eighteen percent (18%) according to (Mayanja *et al.*, 1999; Matee *et al.*, 2000) up to greater than sixty percent (>60%) as reported by (Nweze *et al.*, 2011; Kwamin *et al.*, 2013; Dos Santos Abrantes *et al.*, 2014), for that, use of antifungal agents for both prophylactic and treatment purposes increases (Belazi *et al.*, 2014). Moreover, additional reported cases of *Candida* species showing resistant to azole antifungals were also revealed (White *et al.*, 1998; Fournier, 2011).

This candida disease, oropharyngeal candidiasis (OPC) is one of the most frequent opportunistic fungal infections among HIV-infected individuals (Lattif *et al.*, 2004). According to some records, more than 90% of HIV infected individuals develops this infections some time during the progression of their HIV infection (Samaranayake *et al.*, 2000; Kamiru and Naidoo, 2002). Being an opportunistic infection in HIV-infected individuals, the most common agents of candidiasis are, *Candida albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis* Sant'Ana *et al.*, 2002). Non *albicans Candida* species are also implicated with high rates, as opportunistic pathogens associated with diseases especially in immunocompromised hosts. Typically, OPC occurs when the patients' CD4 counts are ≤ 200 , as such considered as clinical marker of immune failure in HIV/AIDS individuals. Generally, oral candidiasis limits food consumption and lead to weight loss is frequently complicated with esophageal candidiasis which put the well being of HIV infected individuals at more risk (Dunic *et al.*, 2004).

Normally, when management of oral candidiasis is prolonged, that usually leads to development of drug resistant candidiasis and these resulted in reports of emergence of resistance to

antifungal agents in HIV-infected individuals with OPC, this poses problem to treatment with standard doses at standard time duration in some cases, additionally, it was found that, prolonged use of antifungal agents can result in shift in non-*albicans* species associated with recurrent infections (Hamza, *et al.*, 2008)

MATERIALS AND METHODS

Study area and study population

This study was carried out at S. S. Wali, HIV clinic of Aminu Kano Teaching Hospital Kano. The state is situated between latitude 11°30'N and longitude 8°30'E and lies at about 1580m above sea level with total land area of 2076 sqkm (Ado, 2009). Patients that were diagnosed as HIV positive, attending Aminu Kano Teaching Hospital (S. S. Wali), department of Medical Microbiology, were the targeted population in this study.

Sample size determination

The sample size was obtained using the formula stated by (Naing *et al.*, 2004) and calculated using the prevalence of 45% from study conducted by (Ngozi, 2009).

$$n = Z^2 P(1-P)$$

$$d^2$$

Where

n = Is minimum samples size

P = Prevalence of oral candidiasis among HIV patient (Ngozi, 2009)

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

d = allowable error of 5% (0.05)

$$= 1.96^2 \times 0.45(1-0.45)$$

$$N = 380$$

Inclusion criteria and Exclusion criteria

Patients clinically diagnosed as HIV positive, willing to participate and not under any antifungal drug were included. Patients, that were HIV negative and those that were positive but under some antifungal drugs were excluded from the study.

Ethical approval

The ethical approval to conduct the work was obtained from research and ethics committee of Aminu Kano Teaching Hospital, with approval number as follows:

AKTH/MAC/SUB/12A/P-3/VI/2505

Sample collection and processing

Two oral swab samples were collected using sterile cotton tipped swabs, from each patients clinically diagnosed as HIV positive. Before collection, tongue pressure was used to press the tongue gently to swab the sample, as seen in figure 1.



Fig. 1: Effect of oral candidiasis

Sample collection for oral candidiasis

Smear preparation

The cotton tipped swab was used to make smear on the surface of clean, grease free glass slides and allowed to air dry, and fixed over bunsen flame and allowed to cool.

Direct gram staining

The smeared slide was flooded with crystal violet for one minute and rinsed up with water. It was covered with luguols iodine for one minute and rinsed up with water. It was then decolorised briefly with acetone and rinsed up with water before it was counterstained with neutral red for two minutes and rinsed up with water. It was examined under oil immersion objective (Cheesbrough, 2005).

Potassium hydroxide (KOH) wet mount

A dropped of normal saline was added on a clean grease free glass slide, the oral swab samples were emulsified. Ten per cent (10%) KOH was dropped onto the preparation, 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 using 10x and 40x objective lens for characteristic morphology of *Candida* species as can be seen in figure 2.

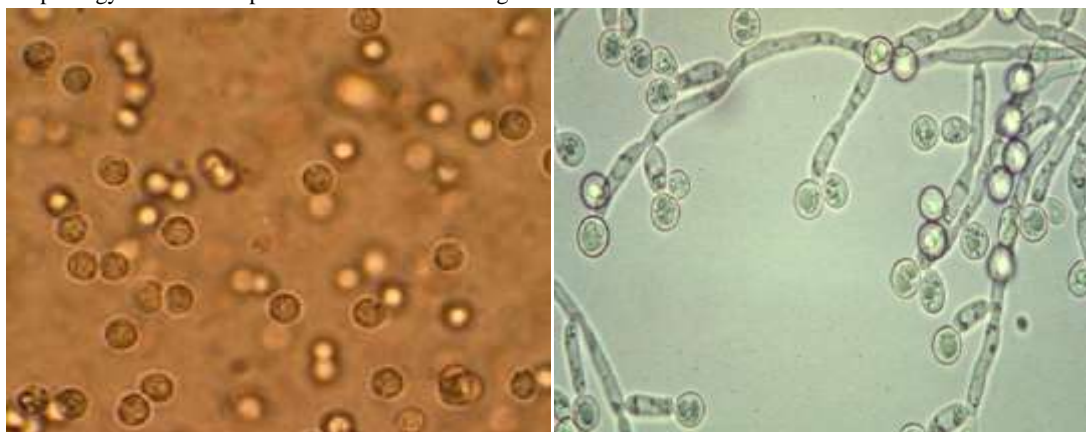


Fig. 2: Microscopic appearance of *Candida* species

Isolation and purification of *Candida* spp from sampled patient

The second swab was inoculated by streak method using wirer loop onto Sabouraud Dextrose Agar (SDA) and incubated in the autoclave at 37°C for 48hr. Isolated colony obtained from overnight growth of the culture was sub cultured onto another

Sabouraud Dextrose Agar (SDA) plates and incubated at 37°C for 48hrs to obtained pure isolates.

Phenotypic characterization of the isolates

The isolates obtained from pure culture were sub cultured onto chromogenic agar and incubated at 37°C for 48hrs.

Germ tube test

About 0.5ml of serum were dispensed into small test tubes, the colonies from pure culture were gently emulsified in the serum and incubated at 37°C for 3 hrs.

A drop of the preparation were transferred to a clean grease free slide and covered with cover slip and focused using 10x objective lens and examined using high power objective at thirty minutes intervals (Ochei, 2002).

Statistical methods

Data generated were entered into microsoft excel and then analyzed using SPSS software (version 20).Level of significance was set at 95% confidence limit (P-Value <0.05)

Results

According to the results one hundred and nine (109) were positive for candida from the oral swab samples, making the prevalence of oral candidiasis to 28.8% while 270(71.2%) had

candida negative cultures (Table1). Among the 109 patients with oral candidiasis, three (3) candida species were isolated and identified phenotypically. These were *Ccandida albicans*72 (66.1%), *Candidaglabrata* 13(11.9%),and *Candidakrusie* 24(22.0%),(Table 2). The result also showed the highest isolation rate of the various *candida* spp were at the age range of 21-30 years, with a total of 32(29.4%) of the total isolates. The rest of the isolates-age group distributions were: 26(23.9%), 18(16.5%), 16(14.7%), 12(11.0%), and 3(2.7%), for age brackets of 31-40, 11-20, 41-50, 61-70 and 51-60 respectively. Accordingly, the age limit of 1-10 had the least isolation rate of 2(1.8%) in the study (Table 3). *Candida albicans* and *Ccandida krusie* were the most isolated oral candida species in the age of 21-30 years. Based on gender distribution, of the 109 positive cultures examined 68(62.4%) were females while 41(37.6%) were males (Table 4).

Table 1: Distribution of oral candidiasis among the enrolled patients

Status	No of patients	Percentage (%)
Positive	109	28.8
Negative	270	71.2
Total	380	100

Table 2: The different *Candida* species isolated

<i>Candida</i> spp	frequency	Percentage (%)
<i>C. albican</i>	72	66.1
<i>C. glabrata</i>	13	11.9
<i>C. krusie</i>	24	22.0
TOTAL	109	100

Table 3: Distribution of the isolates based on age groups

Age groups	Frequency	Percentages (%)
1-10	2	1.8
11-20	18	16.5
21-30	32	29.4
31-40	26	23.9
41-50	16	14.7
51-60	3	2.7
61-70	12	11.0
TOTAL	109	100

Table 4: Sex-wise distribution of the *Candida* infection

Gender	Frequency	Percentage (%)
Female	68	62.4
Male	41	37.6
Total (N)	109	100

DISCUSSION

The study was undertaken to assess the magnitude of oral candidiasis among HIV infected individuals in the study area. A prevalence of 28.8% was obtained in the cause of the study. This is in agreement with report of Sani-*et al.* (2017) in a research conducted on prevalence of oropharyngeal candidiasis among HIV patients attending ART clinic, in infectious disease hospital (IDH), Kano, which was in the same state, and they got 25% as their prevalence rate. However, a higher prevalence, (52.2%) was reported in a Southwestern Ugandan (Ezera agwu *et al.*, 2012) in a study distribution of yeast species associated with oral lesions in HIV-infected patients in Southwestern Uganda.

With regards to the yeast type involved in the disease, three different *Candida* species were isolated during the study, *C. albicans*, *C. galibrata* and *C. krusei*. This finding is almost similar to that obtained by Ezera agwu and co-workers but with additional species observed, in 2012, where they isolated *Candida albicans* and *Candida galibrata* but the authors found no *Candida krusei*, however, they reported two additional species, *Candida tropicalis* and *Candida norvegensis* in their studies (Ezera agwu *et al.*, 2012). This variation could be due to geographical differences between the two study areas.

In this study, *Candida albicans* had the highest (72%) isolation rate as against the rest of the species isolated. But a much lower (33.90%) isolation frequency was observed by Martha *et al.*, 2016), who reported *Candida albicans* as the commonest species detected among HIV-infected individuals. This low detection rate could be attributed to higher level of awareness and health education in the studied community.

For age wise distribution, the age range of 21-30 had the highest 32(29.4%) infection rate during the study. Similar age bracket (26-33) was observed to be most infected in the report of Ndukwe *et al.*, 2016. But a contrasting finding was noticed by Manikandan and Amsath

(2015) and the authors reported 31-60 years age limit as highest (72.7%) percentage prevalence.

With regards to gender, result of the study revealed that, females were more prone to the disease 68(62.4%) than males 41(37.6%). However, the reverse was the case in the study of Sani *et al.* (2017), who reported 20(41.7%) and 10(13.8%) as male and female infection frequencies respectively.

Conclusion

It can therefore be concluded from the result that, *Candida* species are among the major opportunistic pathogens in close association with HIV patients in the study area. The

disease also proved to be of public health concern as one of the most active age group (21-30) years was found to be at higher risk.

RECOMMENDATION

Routine check for opportunistic infections including oral candidiasis is important and should always be carried out among HIV patients in the study area.

Ethical approval

The ethical approval to conduct the work was obtained from research and ethics committee of Aminu Kano Teaching Hospital, with approval number as follows:

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Conflict of interest

The authors want declare that, there is no conflicts of interest in whatever form

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