



SJMLS-2(3)-2017-010

Epidemiology of Oral Candida Infection among People Living With HIV/AIDS (PLWHA) in Bida, Niger State, Nigeria.

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Abstract

Oropharyngeal candidiasis is a common occurrence in the course of Human Immunodeficiency Virus (HIV) disease progression and it is predictive of immunosuppression. Against the background of the paucity of data on the epidemiology of candida infection among People Living With HIV/AIDS (PLWHA) in Bida, Niger State, Nigeria, the study was conducted. The study is a prospective and epidemiological survey carried out on HIV positive patients attending ART clinic in Federal Medical Centre, Bida. Oral swabs were collected and cultured for the isolation of candida and speciation of isolate was done using Chromagar candida. The CD4⁺ cell counts of all the subjects were estimated using the Partec Cyflow Counter 2 and correlated with presence or absence of candidiasis. Out of a total of the 390 HIV positive patients, 116 (29.8%) yielded growth of candida species with the distribution of *Candida albicans* 77(66.4%), *Candida tropicalis* 26(22.4%), *Candida krusei* 12(10.3%) and a mixed growth of *Candida albicans* and *Candida tropicalis* 1(0.9%). There was a statistically significant difference between oral candidiasis (*Candida albicans*) and CD4⁺ count < 50 cells/ μ l ($p=0.0030$). The study however found out that patients with moderate and high CD4⁺ count (>200 to 1000 cells/ μ l) are also susceptible and have the chances of developing oral candidiasis. *Candida albicans* was the most common specie of oral candidiasis among the HIV/AIDS subjects in Bida. There was no statistical significance between oral candidiasis and duration HIV infection. The study revealed that *Candida albicans*, *Candida tropicalis* and *Candida krusei* are the species

causing oropharyngeal candidiasis among People Living With HIV/AIDS. An overall oropharyngeal candidiasis prevalence of 29.8% was observed among the studied population. CD4⁺ count <200cell/ μ l is not a risk factor for acquiring oropharyngeal candidiasis.

Key words: Epidemiology, Oral candidiasis. HIV, *Candida albicans*, Bida-Nigeria

Introduction

Human Immunodeficiency virus (HIV) is the most significant emerging infectious pathogen of the 20th century. Since the Acquired Immunodeficiency Syndrome (AIDS) was first recognized in 1981 in North America, the HIV/AIDS epidemic continues its expansion across the globe (Massur *et al.*, 1981). HIV progressively reduces the effectiveness of the immune system and makes individuals susceptible to opportunistic infections and tumors. The progressive destruction of the immune system by chronic HIV infection leading to progressive fall in level of CD4 cells (<200). The significant reduction in the CD4 helper lymphocyte count is responsible for the occurrence of infections by opportunistic microorganism (Holmberg and Mayer, 1986). The prevalence of opportunistic mycoses has

dramatically increased during the past few years. The etiological agents are otherwise incapable of causing disease in healthy individuals. These opportunistic fungi are potential pathogens seen in the immune-compromised patients (Chungh and Khan, 2000). The most common and some uncommon mycoses seen in patients with HIV/AIDS are Candidiasis, Cryptococcosis, Histoplasmosis, Aspergillosis and Dermatophytes. Other fungal infections that are also seen in HIV/AIDS patients are Coccidioidomycosis, *Blastomycosis penicilliosis* and Sporotrichosis and Pneumocystis pneumonia (Jagdish, 2008). Candida and Cryptococcus are the main fungal pathogens in India, the incidence and species are variable depending upon geographical area and knowledge about the pattern of pathogens is a must in order to serve the HIV infected patients better (Kundnani *et al.*, 2010). Ninety percent of patients develop some form of candida infection at some time of the disease. *Candida albicans* is the most common species but other species like *Candida tropicalis*, *Candida krusei*, *Candida glabrata* are also involved (Gugnami *et al.*, 2003).

Kundnani *et al.* (2010) found Candida in 53.48% of patients suffering from oral candidiasis in India. Out of which 10 gave a history of dyspergia suggesting possible esophageal involvement. This has shown the increasing significance of Candida non-albicans in HIV infected patients. Species identification of Candida becomes more important keeping in mind the inherent resistance of some Candida species to some anti-fungal agents. Other studies have found the incidence of candidiasis ranging from 30- 70% (Shailaja *et al.*, 2004; Agyagri *et al.*, 1999). Candida infections in AIDS cases are usually limited to superficial mucosal involvement. Candidemia occurs rarely (Hage *et al.*, 2002).

There is paucity of data on the on the epidemiology of candida infection among People Living With HIV/AIDS (PLWHA) in Nigeria. The aim of this study was to determine the epidemiology of oral candida infection

among People Living With HIV/AIDS (PLWHA) in Bida, Niger State.

Materials and Methods

Study Area

The study was carried out in the Antiretroviral Therapy (ART) Clinic of Federal Medical Centre Bida, located in Bida Local Government Area of Niger State, North Central Nigeria. Bida is the second largest town in Niger State. It is a local government area in Niger State, Nigeria. The local government area covers an area of 51 square kilometers and has a population of 188,181. Bida is located South-West of Minna the capital of Niger State, about 9km North of River Kaduna, along Bida-Mokwa road (Federal Republic of Nigeria Official Gazette, 2007). There are about 9 government health institutions which include, a Federal Medical Centre (FMC), a general hospital, five primary health care clinics and 2 dispensaries. There are also over ten private health institutions. There are other social amenities in the town like pipe borne water and electricity supply. FMC, Bida is a 200-bed tertiary hospital serving as a referral center for all primary and secondary facilities in and around Niger State.

Sampling Population

Selection of Subjects

Subjects for this study comprised of consenting PLWHA attending ART Clinic of FMC, Bida who were selected randomly for the study.

Inclusion Criteria

Consenting HIV positive patients referred to the ART Clinic constituted the subjects for this study.

Exclusion Criteria.

Non-consenting HIV positive patients and HIV negative individuals were excluded from participation in this study.

Ethical Consideration

Ethical approval for this study was obtained from the Ethical Review Committee of FMC, Bida. The sample collection was explained to



the patient using the information sheet. Each patient was required to give a written informed consent before being eligible to participate in the study.

Sample Size Determination

The minimum sample size for this study was determined using the Fischer formula $n = z^2 pq/d^2$ (Fisher *et al.*, 1998).

n = the desired sample size when population is greater than 10,000

z = the standard normal deviated, usually set at 1.96 (or 2.0) which corresponds to the 95% confidence limit.

p = the proportion in the target population estimated to have a particular characteristic which is 52.5% (Esebelahie *et al.*, 2013).

p = 0.525

q = 1.0 – p

q = 1- 0.525 = 0.475

d = degree of accuracy desired usually set at 0.05

Thus, if the proportion of target population which contains the characteristic is 0.05, the z statistic is 1.96 and the desired sample size will be calculated thus:

$n = 1.96 \times 1.96 \times 0.525 \times 0.473 / 0.0025$

$n = 391$

Data Collection

A semi- structured questionnaire was used to collect necessary data from the 390 recruited subjects for the study. The data obtained included socio-demographic characteristics, duration of HIV infection, use of ART, CD4⁺ counts estimation. Data from collected blood samples was collated and analyzed statistically. The research instrument included data from questionnaire, laboratory investigation, microscopy, culture results and CD4⁺ counts.

Specimen Collection

Depending upon clinical presentation, oral swab for culture and blood for CD4 estimation was

collected from each subject. Five milliliters of whole blood were drawn from the media cubital vein of every subject into EDTA anticoagulated tube for CD4⁺ estimation while the oral swab was collected with sterile swab stick and sent to the laboratory for immediate processing.

Laboratory Investigation

Laboratory analysis was carried out in the Medical Microbiology Laboratory FMC Bida.

CD4⁺ Estimation

The new model Partec Cyflow Counter 2 (Sysmex Partec GmbH Görlitz, Germany) was used for CD4⁺ estimation.

Specimen Analysis

Samples were processed according to standard mycological techniques (Milne, 1996). Each specimen was examined for the presence of fungi by microscopy using wet mounts (KOH and Nigrosin mounts), and stained preparation using (Lacto phenol cotton blue stain). Isolation of the fungus was done using Sabouraud dextrose agar and the plates were incubated at 37^oc for 24 - 72hours. Growth rate, colony characteristics and microscopy was used to identify candida by their characteristic shape and hyphae on wet preparation using Lacto phenol cotton blue. Candida isolates were sub - cultured into agar slope for speciation using Chromagar candida.

CHROMagar candida

Chromagar candida is a special media for isolation and differentiation of major clinical species of candida. It displays high specificity and sensitivity for 3 of the major candida species (*Candida albicans*, *Candida tropicalis* and *Candida krusei*). Candida isolates were identified to species level by culturing on Chrom agar candida. Incubated aerobically at 37^oC at 36- 48hrs. Identification is based on colour production.

Table 1: Identification and interpretation of Candida species based on colour production

<i>Candida Species</i>	Color Production
<i>Candida albicans</i>	Green
<i>Candida tropicalis</i>	Metallic blue
<i>Candida krusei</i>	Pink, Fuzzy
<i>Candida glabrata</i>	Mauve

Statistical Analysis

The data generated from this study was analyzed using the statistical software SPSS version 16 (SPSS Inc. Chicago, Illinois). The statistical testing was used to check correlation of epidemiology of candida infection in People Living With HIV/AIDS. Categorical variables were compared using the chi- square test. A p-value of <0.05 was taken to be statistically significant.

Results

Three hundred and ninety (390) oral swabs were collected among patients with HIV/AIDS population in Bida. Participants in this study were within the ages of 8 months and 70 years with a mean age of 38.02 ± --- years.

The prevalence of oral candidiasis in this study was found to be 29.8% as shown in table 2. Table 3 shows the specie distribution of *Candida albicans* 77(66.4%), *Candida tropicalis* 26(22.4%), *Candida krusei* 12(10.3%) and a mixed growth of *Candida albicans* and *Candida tropicalis* 1(0.9%). Table 4 shows the age distribution of candidiasis among HIV/AIDS patients in Bida. The prevalence of oral Candidiasis in relation to the age of participants showed statistical difference with *Candida albicans* colonization but showed no statistical difference with *Candida krusei* and *Candida tropicalis*. Table 5, shows the relationship between candidiasis and gender of

the participants, out of the overall 116 (29.8%) *Candida* species isolated from this study, 82(28.2%) were isolated from females while 34(31.2%) were found in the male participants while 191(70.2%) had no growth. The difference was not statistically significant (p> 0.05). Table 6 shows the relationship between CD4⁺ counts and oral candidiasis among HIV/AIDS subjects in Bida. CD4⁺ counts with *Candida albicans* shows a p- value of 0.0030 which is statistically significant while *candida krusei* had p =0.9503 and *candida tropicalis* had p = 0.1079. High prevalence of candidiasis was seen in CD4⁺ counts < 50cells 7(53.8%). There was statistical significant difference (p<0.05) between oral candidiasis and CD4⁺ counts of participants in this study. Table 7 shows the frequency of isolates with participants on HAART usage. Out of the overall prevalence of 116(29.8%) participants with oral candidiasis in this study, 110(39. 6%) were on HAART while 6(33.4%) were Naïve to HAART. The difference was however, not statistically significant (p>0.05). Table 8 shows the relationship between oral candidiasis and duration of HIV/AIDS participants. The highest frequency of oral candidiasis was reported in participants with 1-5 years duration of the infection 61(28.0%) while 11 years and above recorded the lowest frequency of 3(30.0%). There was no statistical significant difference (p>0.05) between oral candidiasis and duration of HIV/AIDS.

**Table 2: Prevalence of Oral Candidiasis in HIV/AIDS Patients**

Candida Species	No. of Participants	Percentage
<i>Candida albicans</i>	77	19.7%
<i>Candida tropicalis</i>	26	6.7%
<i>Candida krusei</i>	12	3.1%
Mixed growth (<i>Candida albicans</i> and <i>Candida tropicalis</i>)	1	0.3%
No growth	274	70.2%
Total	390	100%

Table 3: Distribution of Various Species of Candida Isolated

Species of Candida	Number of Isolates	% Numbers of Isolates
<i>Candida albicans</i>	77	66.4%
<i>Candida tropicalis</i>	26	22.4%
<i>Candida krusei</i>	12	10.3%
Mixed growth (<i>Candida albicans</i> and <i>Candida tropicalis</i>)	1	0.9%
Total	116	100%

Table 4: Candida Isolates in Relation to Age of Participants

Age	No growth	<i>Candida albicans</i>	<i>Candida krusei</i>	<i>Candida tropicalis</i>	Mixed growth
0-9 Years (n=36)	22(61.1%)	13 (36.1%)	0(0%)	1(2.8%)	0(0%)
10-19Years (n=16)	12(70.0%)	3(18.7%)	0(0%)	1(6.3%)	0(0%)
20-29Years (n=66)	44(66.7%)	15(22.7%)	1(1.5%)	5(7.6%)	1(1.5%)
30-39Years (n=136)	94(69.1%)	22(16.2%)	5(3.7%)	11(8.1%)	0(0%)
40-49Years (n=92)	62(67.4%)	16(17.4%)	6(6.5%)	8(8.7%)	0(0%)
50-59Years (n=41)	36(87.8%)	5(12.2%)	0(0%)	0(0%)	0(0%)
60-69Years (n=5)	4(80.0%)	1(20.0%)	0(0%)	0(0%)	0(0%)
70 & Above(n=2)	0(0%)	2(100%)	0(0%)	0(0%)	0(0%)
N=390	194(70.2%)	77(19.7%)	12(3.1%)	26(6.7%)	1(0.3%)

Age versus *Candida albicans* p = 0.0139. *Candida krusei* p = 0.3680 *Candida tropicalis* p = 0.5998. n = Numbers of Participants N= Grand total

Table 5: Candida Isolates in Relationship to Gender of Participants

Gender	No Growth	<i>Candida albicans</i>	<i>Candida krusei</i>	<i>Candida tropicalis</i>	Mixed growth
Male (n=109)	75(68.8%)	26(23.9%)	2(1.8%)	6(5.5%)	0(0%)
Female (n=281)	199(70.8%)	51(18.1%)	10(3.6%)	20(7.1%)	1(0.4%)
N= 390	194(70.2%)	77(19.7%)	12(3.1%)	26(6.7%)	1(0.3%)

Legend

n = Numbers of participants

N = Grand total

p =0.963

Table 6: Distribution of Candida Isolates with CD4⁺ Counts of Participants

CD4 ⁺ Count Cells/μl	No Growth	<i>Candida albicans</i>	<i>Candida Krusei</i>	<i>Candida tropicalis</i>	Mixed growth
<50 Cells/μl (n=13)	5(38.5%)	7(53.8%)	0(0%)	1(7.7%)	0(0%)
<100 Cells/μl (n=12)	9(75.0%)	3(25.0%)	0(0%)	0(0%)	0(0%)
100-200Cells (n=47)	30(63.8%)	14(29.8%)	1(2.1%)	2(4.3%)	0(0%)
201-500Cells (n=154)	102(66.2%)	29(18.3%)	5(3.2%)	17(11.0%)	1(0.6%)
501-1000Cell (n=135)	109(80.7%)	17(12.6%)	5(3.7%)	4(3.0%)	0(0%)
>1000Cells (n=29)	19(65.5%)	7(24.2%)	1(3.4%)	2(6.9%)	0(0%)
N=390	274(70.2%)	77(19.7%)	12(3.1%)	26(6.7%)	1(0.3%)

CD4⁺ count versus *Candida albican*, p = 0.0030, *Candida Krusei*, p = 0.9503, *Candida tropicalis*

Legend

p = 0.1079

n = Numbers of participants

N = Grand total

**Table 7: Candida Species Isolated from Participant in Relation to HAART Usage**

Participants	No growth	<i>Candida albicans</i>	<i>Candida krusei</i>	<i>Candida tropicalis</i>	Mixed growth
On HAART (n=372)	262(70.4%)	73(19.6%)	11(3.0%)	25(6.7%)	1(0.3%)
HAART Naïve (n=18)	12(66.6%)	4(22.2%)	1(5.6%)	1(5.6%)	0(0%)
N=390	274(70.2%)	77(19.7%)	12(3.1%)	26(6.7%)	1(0.3%)

HAART usage versus HAART naïve *Candida albicans* p = 0.7868, *Candida krusei* p = 0.5330, *Candida tropicalis* p = 0.8466

Legend

n = Numbers of participants

N= Grand Total

Table 8: Candida Species Isolated from Participants in Relation to HIV Duration

Duration of infection	No Growth	<i>Candida albicans</i>	<i>Candida krusei</i>	<i>Candida tropicalis</i>	Mixed growth
< 1 year (n=48)	26(54.2%)	14(29.1%)	2(4.2%)	6(12.5%)	0(0%)
1-5 years (n=218)	157(72.0%)	39(17.8%)	8(3.8%)	14(6.4%)	0(0%)
6-10 years (n=114)	84(73.6%)	22(19.3%)	2(1.8%)	5(4.4%)	1(0.9%)
11 yrs & Above (n=10)	7(70.0%)	2(20.0%)	0(0%)	1(10.0%)	0(0%)
N = 390	274(70.2%)	77(19.7%)	12(3.1%)	26(6.7%)	1(0.3%)

Duration of HAART versus *Candida albicans* p= 0.3651, *Candida krusei* p = 0.6976, *Candida tropicalis* p = 0.2865

n = Numbers of participants

`N = Grand total

Discussion

The prevalence of oral candidiasis among HIV/AIDS patients in this study was 116 (29.8%). Our observed prevalence is in agreement with a previous report by Enwuru *et al.* (2008) among HIV seropositive patients in Lagos, Nigeria which indicated a prevalence of 34.7%. Our observed prevalence is however higher than that report by Lar *et al.*, (2012) 9.68% in Jos, Nigeria. Our observed prevalence of oral candidiasis is lower than that reported in other studies in Africa by Agwu *et al.*, (2011) in Uganda (52%) and Kwamin *et al.* (2013) (65.5%) in Ghana. Similarly, our observed prevalence of oral candidiasis is also lower than that reported (57.5%) in a previous report in Brazil (Rejare *et al.*, 2002).

Oral candidiasis was highest among age group 30-39 years 38 (31.1%) out of the overall 116(29.8%) prevalence of oral candidiasis found in this study. However, this study found a relationship between age and *Candida albicans* infection ($p=0.0139$) but not with *Candida krusei* ($p = 0.3680$) and *Candida tropicalis* ($p = 0.5998$) in Bida, but it can be deduced that the implication of these age groups with high prevalence are youths which are the worst affected group and should be the target of HIV intervention (Shittu *et al.*, 2013).

This study observed a predominance of oral candidiasis in females (28.2%) to males (31.2%) with a female to male ratio of 2.5:1 oral candidiasis infection. However, there was no significant difference in the prevalence of oral candidiasis based on gender and among HIV positive patients in Bida.

With reference to the immune status of the study participants, it was found that $CD4^+$ count <50 cells/ μ l was a risk factor for acquiring *Candida* colonization among HIV/AIDS patients in Bida. The association between the $CD4^+$ counts of the participants *Candida albicans* infection was statistically significant ($p =0.0030$) while *Candida krusei* ($p =0.9503$) and *Candida tropicalis* ($p = 0.1079$) are not significant. High prevalence of candidiasis was seen in $CD4^+$ counts < 50 cells 7(53.8%). There

was no statistically significant difference ($p<0.05$) between oral candidiasis and $CD4^+$ counts of participants in this study. This finding is supported by a recent observation of Esebelahie *et al.* (2015) in Benin City, who reported significant relationship between low $CD4^+$ counts (< 200 Cells/ μ l) and *Candida* colonization.

The findings in this study provides evidence of candidiasis in HIV- infected patients on HAART and as well as HAART naïve in Bida. There was no statistically significant difference between *Candida* isolates and participants on HAART [*Candida albicans* ($p= 0.7868$), *Candida krusei* ($p = 0.5330$) and *Candida tropicalis* ($p = 0.8466$)]. HIV patients either on HAART or HAART naïve were predisposed to oral candidiasis which agrees well with the study in Jos, Nigeria (Lar *et al.*,2012). In contrast, Cassone *et al.* (1999) reported that, following the introduction of highly active antiretroviral therapy (HAART) there was a reduction in occurrence of opportunistic infections, prevalence of oral manifestations and oral candidiasis. This is surprising since the goal of ART is to reduce viral load with a resultant restoration of immune function and reduced incidence of opportunistic infection. The susceptibility of candida isolates to various antifungal agent can be a challenge. A previous report in Iran found an increased fluconazole resistance in *Candida albicans* and other species among HIV and non-HIV patients (Anwer *et al.*,2012).

Our findings revealed no significant association between oral candidiasis and HIV duration. *Candida albicans* ($p = 0.3651$), *Candida krusei* ($p = 0.6976$) and *Candida tropicalis* ($p = 0.2865$). This study observed a high frequency of *Candida* species isolation in 1-5 years and 6-10 years HIV duration 61(28.0%) and 30(26.4%) respectively and a lower occurrence in < 1 year and 11 years duration of HIV. However, there was no much mention of candidiasis and HIV duration in HIV/AIDS patients in recent literatures.



Conclusion

The study revealed that *Candida albicans*, *Candida tropicalis* and *Candida krusei* are species causing oropharyngeal candidiasis with an overall prevalence of 29.8% in patients with HIV/AIDS attending ART clinic of Federal Medical Centre, Bida. Also, CD4+ count <50 cells/ μ l is a risk factor for acquiring oropharyngeal candidiasis.

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Citation: Emumwen Ehiosu Favour, Ogefere Helen Oroboghae, Omosigho Omoruyi Pius, Emumwen Egbaziegbere Gidion (2017). Epidemiology of Oral *Candida* Infection among People Living With HIV/AIDS (PLWHA) in Bida, Niger State, Nigeria. *Sokoto Journal of Medical Laboratory Science*; **2(3)**:75 – 84.

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