

Risk Factors Associated With Oral Cavity Colonization By *Candida Albicans*, Among Immunosuppressed Persons In Uyo, South South Nigeria

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ABSTRACT

Candida infection has in recent times gained considerable attention globally as a result of its association with increasing incidences of immunosuppressive disease conditions in addition to other human-related risk factors. The aims of this study were to assess the prevalence of oral cavity colonization with *Candida albicans* among immunosuppressed individuals and to assess probable risk factors that could influence oral cavity colonization. The study examined 120 single mouth swab specimens from HIV-infected patients (n=17) and diabetics (n=21) attending the out-patient clinic of University of Uyo Teaching Hospital, Uyo-Nigeria and apparently healthy hospital staff and students (n=82) used as control. Informed consent was obtained from all subjects; in the case of juveniles, from their parents. The mouth swabs were cultured on Sabouraud dextrose agar, incubated at 37°C for 48hrs and *Candida albicans* identified using Tween 80 opacity test response. HIV serostatus of patients was determined using the Immunocomb Enzyme Immunoassay and Immunoconfirm HIV test kits. Forty two (35.0%) of the subjects were detected with oral cavity colonization by *Candida albicans*. The colonization rates were not significantly different among males and females ($p > 0.05$). HIV sero-positive status, diabetes mellitus (DM), but not advancement in age and recent consumption of broad spectrum antibiotics were significantly associated with higher colonization rates ($p < 0.05$). The Odds Ratio evaluated for these risk factors at 95% confident interval were 3.02(1.02-8.97), 2.67(1.00-7.18), 0.45(0.165-1.18) and 1.88(0.763-4.45) respectively. Apparently healthy subjects were by far less colonized by *Candida* than the non-healthy population, $p < 0.014$. Study findings revealed that although the overall prevalence of oral cavity colonization by *Candida albicans* was relatively low, apparently healthy subjects were by far less colonized by *Candida albicans* than the non-healthy populations, especially the HIV infected and those having diabetes disease.

Key words: *Candida albicans*, Colonization, Risk factors.

INTRODUCTION

Candida infection has in recent times gained considerable attention globally as a result of its association with increasing incidences of immunosuppressive disease conditions in addition to other human-related risk factors. *Candida* species are ubiquitous yeasts and common residents of mucosal surfaces of the human oral cavity, the gastrointestinal and urogenital tracts (1-4). It can be present in specimens as a result of environmental contamination, colonization or actual disease processes (5-6). *Candida* spp. that colonized the oral cavity are the sources of the yeasts that cause

oral candidiasis (7). Studies have shown that oropharyngeal candidiasis is the most prevalent opportunistic infection in individuals infected with human immunodeficiency virus (8,9,10). Susceptibility to opportunistic infection among these patients is directly related to their depressed immune state (8). Mucocutaneous forms of candidiasis are often related to defects in cell mediated immunity, while systemic spread is generally associated with neutropenia (2,4,11). As a result of increasing numbers of immuno-compromised individuals within the human population, the incidence of *Candida* infections

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have increased dramatically in the last decade (4,6). Among the species of *Candida*, *C. albicans* is the most prevalent causative agent of candidiasis and constitutes the fourth most common nosocomial blood stream isolate in gastrointestinal tract and the genito-urinary system (7,8,9). *Candida* is a commensal organism and part of the normal flora in about 30%-50% of the population. It is capable of producing opportunistic infections within the oral cavity when appropriate predisposing factors exist. Axel (12) identified three general factors that may favour clinically evident oral candidiasis. These factors are: the immune status of the host, the oral mucosal environment and the particular strain of the *Candida albicans*. Those factors that may alter the oral cavity environment include antibiotic therapy, poor oral and dental hygiene, malnutrition and heavy smoking. The specific conditions which may predispose a person to develop oral candidiasis in relation to the immune status of the patient include: old age, infancy, radiation therapy, HIV/AIDS, diabetes mellitus, chronic renal failure, thyroid disorders, respiratory infections, severe iron deficiency and artificial dentures, as well as malignancies, etc. The aim of this study was to determine the prevalence of oral cavity colonization by *Candida albicans* among out-patients in a tertiary health facility and to relate the colonization rates with specific predisposing factors.

MATERIALS AND METHODS

Subjects

This case-control (non-matched) study was conducted between April and June 2008. Oral cavity swab samples were randomly collected from a total of 120 subjects aged between 15 and 75 years of both sexes. The subjects were stratified into four groups: HIV positive subjects who were anti-retroviral naïve ($n=17$); subjects that attended the diabetes clinics ($n=21$); and subjects irrespective of health status who reported recent broad spectrum antibiotic consumption (within the last 3 months prior to study), $n=45$, were

included in the study. Hospital staff and students ($n=82$) with no reported illness as at the time of screening were used as control. Information on HIV status, diabetes mellitus disease, age and antibiotics consumption were obtained from the subjects during sample collection. This included apparently healthy control subjects. All the HIV positive subjects were retested with the Immunocomb II HIV 1 & 2 Biospot (ORGENICS Ltd, Israel) enzyme immunoassay test and confirmed with Immunoconfirm kits (ORGENICS Ltd, Israel), while the diabetics were selected if their fasting blood sugar record was above 7.5 Mmol/l.

Exclusion Criteria

HIV positive subjects receiving antiretroviral drugs as at study period and those whose antibiotic consumption was inconsistent with study criteria were excluded from this study. Also excluded were patients with chronic renal disorder, thyroid disorder and features of iron deficiency.

Sample Collection and Culture

Samples were obtained by swabbing the palatine mucosa and dorsal tongue with sterile cotton wool swabs. These materials were then cultured aerobically on Sabouraud dextrose agar (SDA) plates at 37°C for 48hrs. Cultures were inspected on daily basis for growth of yeast; pure cultures were prepared and stored on agar slopes at room temperature for identification purposes.

Identification of Isolates

All the isolates were identified by germ tube test, chlamydospore production on corn meal agar supplemented with 1% Tween 80 and production of halo or visible opacity response around the inoculation site as described by Slifkin (13). Other confirmatory tests carried out were sugar and nitrogen assimilation tests.

Statistical Analysis: The chi square test was used to determine the level of oral cavity colonization among those with the factor compared with

those without the factor at p value ≤ 0.05 using SPSS statistical package version 17.0. The Odds Ratio (OR) was determined at 95% confidence interval.

Ethical Clearance

The local ethical review committee of the hospital approved the study and informed consent was sought and obtained from all the subjects before included in the study.

RESULTS

In this study, the frequency of isolation of *Candida albicans* from oral cavity of the subjects was 35.0%. *Candida albicans* was the only *Candida* species encountered in this study. There was a tendency towards increasing isolation rate as the subject age increased (Table 1). The highest colonized age group were those of 50 years and above.

In table 2 is presented the distribution of oral cavity colonization by *Candida albicans* among male and female subjects studied. The colonization rate among male (32.2%) and female (37.7%) was not significantly different ($p > 0.05$).

The association of *Candida albicans* colonization of the oral cavity with various possible predisposing factors is presented in Table 3. The predisposing factors considered in this study were: HIV serostatus, diabetes mellitus, and consumption of broad spectrum antibiotics within the last three months before the study.

The prevalence of oral cavity colonization among HIV sero-positive subjects (64.7%) was significantly higher than that among the HIV sero-negative subjects (30.1%), $p < 0.05$ having Odds Ratio (OR) of 3.02 (95%CI 1.02-8.97).

Among diabetics, the frequency of isolation was 61.9% compared to 26.3% among the non-diabetics. There was a significant association between *Candida* colonization and diabetes, OR = 2.67 (95%CI 1.00-7.18), $p < 0.05$.

Although the frequency of *Candida*

colonization seems to be increasing with age, no statistical significant difference and low associated risk was observed between those aged less than 50 and those 50 years and above, $p > 0.05$; OR = 0.45 (95%CI 0.165-1.18). Isolation rate among subjects (irrespective of health status) associated with recent consumption of broad spectrum antibiotics were 40.0% and 26.7% for antibiotic consumers and non-consumers respectively with OR, 1.88 (95%CI 0.76-4.45). Moreover, no statistical significant association was observed between *Candida* colonization and consumption of broad spectrum antibiotics among the subjects studied ($p > 0.05$).

Generally, the frequency of oral cavity colonization among the non-healthy subjects was highly significant compared to the apparently healthy control population; $p < 0.01$, OR = 2.82 (95%CI 1.36-5.86).

DISCUSSION

In this study, the oral cavities of 35.0% of the subjects were found to be colonized by *Candida albicans*. This finding is relatively low but compares favourably with reports of other studies where colonization rates of 30%-50% have been reported among healthy populations in the developed countries (12, 14) where the use of artificial dentures is common. Asymptomatic carriage of *Candida albicans* has been reported to be common among HIV sero-positive patients (15). Findings in this study show that the colonization rate among the subjects that were HIV sero-positive was 64.7%. This finding corroborates that documented by Costa *et al.* (16), and Gugnani *et al.* (17) where oral yeast carriage rates of 62.6% and 65.3% respectively were reported among HIV patients. Similarly, Campisi *et al.* (18) and Sanchez-Vargas *et al.* (19), reported carriage rates of 61.9% and 66.7% respectively among HIV/AIDS patients. Fong *et al.* (20) have reported that persistent asymptomatic carriage of *Candida albicans* was a risk factor for subsequent systematic infection in the patient. A number of studies (21,22, 23) carried out in different parts of Nigeria have reported similar findings. Physicians practicing

in Nigeria, especially in rural areas, should suspect primary HIV infection in all cases of oropharyngeal candidiasis.

The result of this study shows that, a total of 21 of the 120 subjects were diabetics and the rate of isolation of *Candida albicans* from their oral cavities was 61.9%. This finding was highly significant when compared with an isolation rate of 26.3% in non-diabetic subjects. Although candida colonization in diabetics is a matter of debate, It is worthy of note that diabetes mellitus is one of the conditions that can alter the immune status of humans. This therefore implicates diabetes mellitus as a putative predisposing factor for oral cavity colonization by *Candida albicans* in our study population. Oropharyngeal candidiasis can invariably result in systemic candidiasis which could pose serious challenge to therapy compared to treatment of localized oral growth.

It has generally been assumed that advanced age represents a predisposing situation for oral cavity colonization by *Candida albicans* (24). Lockhart *et al.* (25) reported that the frequency of and intensity of oral cavity colonization by *Candida albicans* increased as a function of age independent of denture use. In this study, the lowest colonization rates of 22.5% was reported among those in the age bracket of 30 years and below while, up to 49% was reported in the age bracket of 46 years and above. However, statistical evaluation of the situation indicates no significant difference among those within the age bracket less than 50 years and those above 50 years even as colonization frequency was observed to increase with increase in age. Also, although no significant association was reported in this study among broad spectrum antibiotics consumers, the fact that odds ratio among this group of individuals was almost 2.0 suggests that an increase colonization may risk exist among this sub-population.

In conclusion, the overall prevalence of

oral cavity colonization observed among both healthy and unhealthy seems to be relatively low. However, in comparison, apparently healthy subject were by far less colonized by *Candida* than the non-healthy populations. This study has further strengthened the fact that HIV infection and diabetes mellitus can be strongly implicated as putative risk factors for oral cavity candidiasis in this area. This finding is hoped to provide baseline information that will stimulate further research into the dynamics of oral and systemic colonization by *Candida* species vis-à-vis contribution of other *Candida* species known to cause opportunistic infections (26) among vulnerable populations.

Table 1: Frequency of isolation of *Candida albicans* from the oral cavity according to age groups of the subjects

Age groups (in year)	No. tested	Frequency Isolation (%)
< 20	13	2 (15.4)
21-25	14	3 (21.4)
26-30	13	4 (30.8)
31-35	14	4 (28.6)
36-40	16	5 (3.3)
41-45	11	5 (45.5)
46-50	13	6 (46.2)
> 50	26	13 (50.0)
Total	120	42 (35.0)

Table 2: Frequency distribution of oral cavity colonization in relation to gender

Gender	No. tested	No. (%) Frequency
Males	59	19 (45.2)
Females	61	23 (54.8)
Total	120	42 (35.0)

p-value > 0.05

Table 3: Association of oral cavity colonization by *Candida albicans* with some risk factors

Risk factor	No. tested	No. positive (%)	Odds Ratio (95% confidence interval)	p value Fisher's Exact test (One-sided)
HIV Infection	17	11(64.7)	3.02(1.02-8.97)	< 0.05
Diabetes mellitus	21	13(61.9)	2.67(1.00-7.18)	< 0.05
Age				
< 50 years	94	29(30.9)		
≥ 50 years	26	13(50.0)	0.45(0.17-1.18)	> 0.05
Consumption of Broad spectrum antibiotics among patients and control subjects	75	30(40.0)	1.88(0.76-4.45)	> 0.05

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