Oral Candida carriage among HIV infected and non-infected individuals in Tikur Anbesa specialized hospital, Addis Ababa, Ethiopia

Berhanu Yitayew*, Yimtubezinash Woldeamanuel*, Daniel Asrat†, Admasu Tenna

ABSTRACT
Oropharyngeal candidiasis (OPC) and oral Candida carriage are common problem in HIV-infected populations. Early detection of oral carriage of Candida species is important for identification of patients with the tendency for rapid progression of HIV infection since oral carriage may influence the development of clinically significant candidiasis in these immunocompromised patients. This study investigated the prevalence and level of oral Candida carriage rate among HIV-infected and non-infected individuals in Tikur Anbessa Teaching Hospital, Addis Ababa, Ethiopia. Oral rinse sample was collected from 71 HIV infected and 50 HIV non infected individuals. Out of the total 121 study participants 85(70.2%) were females and 36(29.8%) were males with male to female ratio of 0.4:1. It was found that 66(54.5%) of the study participants were carriers of oral Candida species from which 49(74.3%) were HIV positive. Oral Candida carriage rate among HIV infected participants was 49(69%) where as in HIV non infected participants it was 17(34%). Six Candida species were identified; C. albicans was the predominant Candida species accounted for 53(80.3%), followed by C. parapsilosis 5(7.6%) and three samples revealed with more than one Candida species. Mean colony density of Candida in HIV positive and HIV negative study participants were 2,145.68±3395.12 CFU/ml and 684.71±1941.520 CFU/ml respectively. In conclusion, not only oral Candida carriage but also the density of Candida colony was higher in HIV infected than non infected populations. C. albicans was the predominant species followed by C. parapsilosis.

Keywords: Ethiopia, HIV, Oral Candida Carriage

INTRODUCTION
According to the 2011 World Health Organization (WHO) report, around 34 million people worldwide were living with HIV/AIDS. Oral manifestations of HIV infection have had a significant role in the morbidity and mortality of HIV seropositive patients. Oral manifestations of HIV infections are sometimes the first sign of the infection and often indicate its progression to AIDS. Oropharyngeal candidiasis is (OPC) a common problem in HIV-infected populations caused by yeast of the genus Candida and particularly Candida albicans. Despite the vast improvement in global oral health, problems still continue in many communities and populations around the world. The distribution and severity of oral disease vary in different parts of the world and within the same country or region. In African oral diseases continue to be a major public health problem. Asymptomatic oral Candida carriage also has been shown to be much more common in HIV-seropositive
subjects than in healthy subjects. Some authors have suggested that high density of oral colonization by Candida predisposes to oral candidiasis. Therefore, early detection of oral carriage of Candida is seen to be important for identification of patients with the tendency for rapid progression of HIV infection and also the treatment of Oral Candidiasis (OC). There is different geographical distribution of oral Candida carriage rate and different scholars report different carriage rates.

<table>
<thead>
<tr>
<th>Country</th>
<th>Candida Carrier Rate</th>
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<tbody>
<tr>
<td>Brazil</td>
<td>HIV Positive: 42.43%</td>
</tr>
<tr>
<td></td>
<td>HIV Negative: 7.1%</td>
</tr>
<tr>
<td>Portugal</td>
<td>54.6%</td>
</tr>
<tr>
<td>India</td>
<td>27.9%</td>
</tr>
<tr>
<td>Italy</td>
<td>63%</td>
</tr>
<tr>
<td>Beijing, China</td>
<td>28.6%</td>
</tr>
<tr>
<td>South Africa</td>
<td>81.3%</td>
</tr>
</tbody>
</table>

*Not Determined

Despite the potential clinical relevance of C. albicans, little is known about carriage patterns of C.albicans and NAC species among HIV infected and non-infected populations. It has been suggested that low CD4+ lymphocytes count and high plasma HIV RNA levels significantly correlate with oral Candida carriage. However, there are controversies about association of CD4+ cell count and viral load with asymptomatic oral Candida carriage. A study by Fong et al. (1997) found a correlation between asymptomatic oral Candida carriage, development of thrush, and low CD4+ cell count in HIV+ patients. Campisi et al. (2002) reported that in AIDS patients there was more oral Candida colonization than HIV non-infected subjects, but this characteristic was not associated with CD4+ cell count or viral load. There is no phenotypic difference between the commensal Candida species and that causes oral candidiasis. For example the study in Ethiopia showed that the isolated C.albicans from symptomatic OC HIV positive individual was identical to C.albicans from HIV negative asymptomatic to OC individuals.

According to UNAIDS, Ethiopia has an estimated 1.1 million people living with HIV/AIDS and 90% of HIV-infected individuals suffer at least one episode of oral candidiasis during the course of their disease. Oral candidosis was not focused as a serious health issue in Ethiopia, however, it is the second predominant lesion found to be in HIV infected population next to dental carries. Although there are some studies on the oral Candidiasis in HIV infected Ethiopians, there is a gap of information how the asymptomatic oral Candida carriage distribution in HIV infected patients in comparison with non-infected individuals. So, in an attempt to answer this question the current study was conducted to evaluate Candida species diversity in the oral cavity of HIV infected and non-infected study groups to determine whether the oral Candida colonization in these two groups differs and its association with CD4+ cell count and antiretroviral therapy.

**METHODS**

A hospital based comparative cross-sectional study was conducted from November/2011 to June/2012. It comprises a total of 121 study participants, 71 HIV infected and 50 HIV none infected. The exclusion criteria were oropharyngeal candidiasis, angular chilits, taking antifungal treatment within the last three months of data collection, known diabetes mellitus and proven pregnancy. Screening of study participants for HIV was done using the KHB test (Shanghai Kehua Bio-Engineering Ltd, Shanghai, China; 2008). A positive sample by KHB was retested using the STAT-PAK test (Chembio Diagnostic System Inc, Medford, NY, USA; 2008) and discordant tests by KHB and STAT-PAK were confirmed by Unigold (Trinity Biotech plc, Ireland). CD4 cell

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**Table 1: Reports of Candida Carrier Rate in HIV Positive and HIV Negative Subjects in Different Areas**

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*Not Determined*
The number of HIV positive participants was measured by flow cytometry. Oral rinse sample was collected from study participants. The study participants were instructed to rinse their mouth with 10 ml of sterile phosphate-buffered saline (0.01 M, pH 7.2) for 1 minute and then spit in to a sterile container. The collected samples were centrifuged before inoculation on Sabouraud Dextrose Agar (SDA) (BBL, USA). To allow pure growth of fungi 0.05gm of Chloramphenicol was added to every litter of SDA medium. The collected oral rinse samples were concentrated (10-fold) by centrifugation and then the supernatant was discarded and the pellet resuspended in 1ml of sterile PBS. One hundred micro liters of concentrated oral rinse was inoculated onto SDA (BBL, USA) and incubated at 37°C for 48 hours. Candida species were identified by using germ tube test and API Candida (BiomerieuxRSA France).

All data were analyzed with the Statistical Package of Social Science (SPSS, version 16, USA) program and used to determine the p-values. In all tests, the differences in data were considered statistically significant if the p-values were <0.05. The study had been approved by the Microbiology, Immunology and Parasitology Department Research and Ethical Review Committee (DREC), as well as the Internal Medicine Department Research and Ethical Review Committee, and written consent was obtained from all study participants.

RESULTS

In the current study a total of 121 study participants in two groups were investigated for oral Candida carriage with age range of 19-70 years old. It comprised 29.8% male and 70.2% of female participants. The oral Candida carriage rate in HIV infected individuals is greater than healthy participants. The HIV infected participants were at risk to be colonized by oral Candida (OR=4.32) than HIV non-infected group (Table 2).

<table>
<thead>
<tr>
<th>HIV Sero status</th>
<th>Candida Culture Result</th>
<th>Total No (%)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>HIV Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>49 (69)</td>
<td>22 (31)</td>
<td>71 (100)</td>
<td>4.32 (1.99 – 9.35)</td>
</tr>
<tr>
<td>Negative</td>
<td>17 (34)</td>
<td>33 (64)</td>
<td>50 (100)</td>
<td>1.00</td>
</tr>
<tr>
<td>Total</td>
<td>66 (54.4)</td>
<td>55 (45.5)</td>
<td>121 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Among the total 71 HIV infected study participants 39(54.9%) were on ART and the rest 32(45%) were not on antiretroviral therapy. Out of ART users 26(66.7%) were oral Candida carriers whereas among naïve ART study groups 23(71.9%) were found to be carrier of oral Candida. Although there was no statistically significant difference between oral Candida carriage and no oral carriage with respect to antiretroviral therapy (P=0.64), the carriage rate of non-ART user was greater than among ART users. Among the total 39 ART users 17(43.6%) were short ART users and 22(56.4%) were long ART users (long ART users are those individuals who have used ART regimen for less than 3 years where as long ART users are greater or equal to 3 years). Seven (42.2%) of short and 19(86.4%) of long ART users were oral Candida carrier and there was statistically significant difference between being short and long ART users in respect to oral Candida carriage (P=0.005).

Out of the total 71 HIV infected participants, 15 patients had CD4+ cell count less than 200 Cells/µL, of whom 9(60%) were oral Candida carrier, 37 patients had CD4+ cell count 200-499 Cells/µL, of whom 24(64.9%) were oral Candida carrier, 19 patients had CD4+ cell count greater or equal to 500 Cells/µL of whom 16(84.2%) were oral Candida carrier.

Among the total 121 study participants 49(40.5%) complained of dry mouth. Out of these 40 (81.6%) were HIV positive and the remaining 9(18.4%) were...
HIV negative. Thirty seven (75.5%) of those with complaints of dry mouth and 29(40.3%) with no complaint were oral Candida carriers (P=0.003). The oral Candida colony count also performed to measure the differences among HIV/AIDS infected and non-infected study participants.

| Table 3 Oral Candida colony count in HIV/AIDS infected and non-infected subjects |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| **HIV Sero status**            | 1-1000          | 10001-5000      | 5001-10,000     | >10,000         |
| Positive                       | 31 (63.3%)      | 12 (24.5%)      | 3 (6.1%)        | 3 (6.1%)        |
| Negative                       | 15 (88.2)       | 1 (5.9%)        | 1 (5.9%)        | 0 (0%)          |
| Total                          | 46 (69.7%)      | 13 (19.7%)      | 4 (6.1%)        | 3 (4.5%)        |

**DISCUSSION**

The rate of oral Candida carriage in HIV infected participants was significantly higher than in the non-infected group (69% and 34% respectively)(p-value 0.0001). Being HIV positive had 4.3 times more risk of developing oral Candida colonization than being HIV negative (OR=4.3). The rate of oral Candida carriage in HIV infected individuals is comparable to the rates reported for Brazil (62.6%)6, North Karnataka (71.53%)26, Italy (63%)32 and Argentina (72.3%)27. However, this study revealed slightly lower oral Candida carriage rates when compared to those reported for South Africa (81.3%)9, but higher than the rates reported for India (27.9%)31, Brazil (42.3%)9 and China (28.6%)33. Variations in colonization rates could be caused by the use of different methods for sample collection and processing of specimens (different sensitivities), as well as by the populations studied (different colonization burden due to different geographical location).

It is established that diminished host defense in HIV infection, which is a multifactor process including local and systemic causes, supports the colonization of commensal Candida.28 The low colonization rates with candida in healthy individuals are comparable to those reported for North Karnataka (33.07%)26 and Argentina (39.8%)27. They are, however, lower than the rates reported for South Africa 63% and Boston, USA (57%)29 and higher than those reported for Beijing, China (18%)33, India (22.4%)30 and (11%)31, Brazil (7.1%)4.

C. albicans found to be the most commonly isolated species in all study groups with an overall frequency of 80.3% of single isolate which is comparable with previously done study in Portugal (79%)30, North Karnataka (73.48%)36 and South Africa (70%)8. However, it is greater than the finding in Brazil (38.46%)9 and Italy (56.5%)12. The rest 19.7% of the isolates were NAC and mixed Candida species. More than one Candida species was isolated from three samples. Among the total isolates 4.6% were mixed Candida species (C.parapsilosis plus C.albicans, C.guillirmondi plus C.albicans and C.famata plus C.albicans). This mixed Candida species were isolated only from HIV infected individual which is in agreement with the report from India31 but in contrast with the study by Jabra-Rizk et al.(2003)32, Sullivan et al.(1999)33 and Luqueet al.(2008)27 who isolated mixed Candida isolates from both HIV infected and non-infected populations. This co-existence of Candida species in the mouth cavity may predispose to recurrent oral candidiasis.34 None of the study participants in this study were populated by more than two Candida species which is in contrast with the report from Argentina by Luque et al. (2008)27.

The non-Candida albicans species include C. tropicalis (1.5%), C.guillirmondi (3%), C.parapsilosis (7.5%), C. famata (1.5%) and C. kefyr (3%). Different scholars reported different species distribution in different areas; this is due to different geographical distribution of Candida species. For instance in Brazil C. dubliniensis(5.7%), C. glabrata(1.9%), and C. tropicalis(1.9%)(C. albicans plus C. dubliniensis, C. albicans plus C. glabrata, and C. albicans plus C. tropicalis)9, in north Karnataka C. tropicalis, C. krusei26, ItalyC.krusei4.3%, C. tropicalis 2.2% and C. dubliniensis 2.2%52, South Africa C. glabrata, C.
tropicalis, C. krusei, C. parapsilosis, and C. dubliniensis of non albicans Candida species were isolated. C. parapsilosis was the most commonly isolated NAC species, accounting for 7.5% of the total isolates, which was in agreement with the report by Pomarico et al. (2010) and Torres et al. (2002) and in contrast with the report by Isogai et al. (2010) who found C. tropicalis was the most frequently isolated NAC.

In the present study significant correlation was not found between oral carriage and CD4+ cell count (P >0.05). This finding was in agreement with the previous study done by Ananthalakshmi et al. (2011), however they reported the significance association of CD4+ cell count and oral candidiasis. The current finding is in contrast with the study by Liu et al., (2006) who reported the oral Candida carriage rate is increased when CD4+ cell count was less than 200 cell/µl. However, as the CD4 cell count decrease the level of oral Candida count increase. This is comparable with the report by Lalith et al. (2012) who showed high colony density with low CD4 count which predisposes the carrier to develop oral candidiasis.

Significant correlation was not found between the status of Candida carrier and antiretroviral therapy in the current study. This could be due to characteristics of isolates studied. All the isolates were considered commensal Candida species and not infecting strains, so producing low levels of SAPs and thereby reducing the potential target for the antiretroviral agents. This finding supports the idea that strains of C. albicans isolated from patients with candidiasis have higher SAPs levels than strains isolated from asymptomatic carriers. Short term use of HART had lower risk of being colonized by Candida species with significance correlation. This indicates that long HART use affects the oral health status which is comparable with the report by Nittayananta et al., (2010) who reported that oral health of HIV infected subjects was improved with short-term use of HAART and long-term use of HAART seemed to have adverse effects on oral health status of the subjects.

Among the total study participants 40.5% were complaining of dry mouth out of which 81.6% were HIV infected study participants. From those who were complaining of dry mouth 75.5% were found to be oral Candida carrier. This could be associated with hyposalivation or other perioral problems which reduces the capability of the mucus membrane to remove Candida. The report by Jorge et al. (1993) showed hypo salivation has been associated with increased oral carriage rates of Candida, found a significant correlation between Salivary Flow Rates (SFRs) and Candida counts in patients with underlying systemic diseases and hypo salivation has been associated with increased oral carriage rates of Candida (Navazesh et al., 1995). This finding is in line with the report from Brazil among xerostomia patient (67.9%) and India 70%31. The colony density of Candida in those who complained of dry mouth was 3003.2 CFU/ml (+4391.44) it is slightly greater than non-complaint which was 1055.65 CFU/ml (+1895.82). This finding is greater than the colony count reported from Brazil which was 500 CFU/ml12; this difference could be study participant’s health status and the method of sample collection. They used Chewing-stimulated whole saliva method for sample collection for microbiological investigation which has low sensitivity than oral rinse.41

An attempt was made to enumerate CFU of Candida present in the oral rinse samples collected from the two different groups to know the significance of the count. More number of oral Candida carriers had a colony density of 1-1000 CFU/ml (69.7%) and 19.7%, 6.1% and 4.5% of Candida carrier study participants had Candida colony of 1001-5000 cfu/ml, 5001-10,000 CFU/ml and >10,000 CFU/ml respectively. The mean level of oral Candida colony count in HIV infected group was higher than HIV negative group which was 2145.68 cfu/ml (+3395.12) and 684.71 CFU/ml (+1941.520) respectively. This finding indicates that even though commensal Candida was present in healthy population their level is significantly lower than HIV infected populations. Most of HIV non-infected Oral Candida carriers had a colony density between 1-1000 CfU/ml (88.2%). Among HIV infected oral Candida carrier study participants 6.1% had colony count >10,000 and none of the control carried this large colony. This is in agreement with the report by Patel et al. (2006) who reported 14% of HIV positive had this large number of colony, but none of
HIV negative individuals carried this large number of colony. The finding is also comparable with the report by Lalithet al.(2012) who reported high colony density among HIV infected study group (5925 CFU/ml (+4380), 3325cfu/ml (+6426) and 295CFU/ml (+628.19) for HIV infected who were naïve for ART; HIV infected who were on ART and healthy individuals respectively).

CONCLUSION
As a conclusion the oral Candida carriage is more prevalent in HIV infected populations, but additional studies are important to see the strain variation and antifungal susceptibility pattern of Candida species from symptomatic and asymptomatic individuals.

ACKNOWLEDGEMENT
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