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RESEARCH ARTICLE

STUDY OF CANDIDA COLONIZATION AND SPECIATION IN HIV-POSITIVE PATIENTS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Progressive cell-mediated immunodeficiency with decrease of CD4+ lymphocyte count to less than or equal to 200 cells/mm³ is a major risk factor for colonization with *Candida* species and development of candidiasis. This study was done to investigate *Candida* colonization, speciation and their correlation with CD4+ cell counts in HIV-positive patients on antiretroviral therapy (ART)

Methodology: A prospective, cross sectional study in HIV-infected patients receiving ART. In total, 500 HIV-positive patients on ART treatment and 100 seronegative controls were enrolled in the study. All HIV patients underwent clinical examination and were subjected to CD4+ cell counts. Oral rinse samples were obtained and processed. Identification of *Candida* species was performed by conventional methods.

Results: In the study group of 500, 166 (33.3%) had *Candida* colonization and were culture positive and in 100 healthy controls 25 (25%) of these individuals were culture positive. *Candida* species were isolated in 166/500(33%) and 25/100 (25%) of the HIV-positive subjects and controls respectively. *Candida albicans* was the most frequently isolated species. Patients with CD4+ cell counts \leq 200 cells/mm³ were significantly ($p < 0.0001$) more frequently colonized 39/70(56%).

Conclusion: *Candida* colonization occurs more frequently in HIV-positive patients with CD4+ cell counts \leq 200 cell/mm³. ART significantly reduces OPC. *C. albicans* is the most frequently isolated species. Colonization, non-candida species are also emerging.

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INTRODUCTION

Colonization of the mouth by *Candida* species has a long recorded history. Hippocrates, as early as 377 BCE, reported oral lesions that were probably caused by *Candida* (Odds, 1988). Many yeast species have been isolated from the oral cavity. The majority of isolates are *Candida*, and the most prevalent species is *Candida albicans* (Cannon et al., 1995). In fact *C. albicans* has been demonstrated to be a frequent colonizer of the oral cavity in nearly 75 % of the humans (Ghannoum et al., 2010). In immunocompromised patients, *C. albicans* and other *Candida* species can cause a multitude of disease manifestations ranging from mild oral disease to disseminated candidiasis. Systemic candidemia is recognized as an important though uncommon cause of mortality (Patel et al., 1996). As a result, diagnosis and treatment of disease caused by *Candida* is especially important in HIV/AIDS

patients who, despite the initiation of antiretroviral therapy (ART), continue to suffer from *Candida* associated lesions (Delgado et al., 2009; Thompson et al., 2010). Amongst this spectrum of disease, of particular concern is oropharyngeal candidiasis (OPC), an opportunistic infection of the oropharynx which develops in nearly 90% of Human Immunodeficiency Virus (HIV) infected individuals during the course of their disease (Samaranayake and Holmstrup, 1989; Samaranayake, 1992). CD4+ counts below 200/mm³ or a high viral load ($>10,000$ copies/ml) is a major risk factor for development of OPC in such patients (Samaranayake and Holmstrup, 1989). Recurrent episodes of OPC are seen in HIV/AIDS patients and they are also more prone to develop invasive candidiasis. OPC is generally managed by judicious use of fluconazole. Multiple courses of antifungal drugs, in these patients contribute to antifungal drug resistance. Often antifungal agents are also less efficacious and take a longer time to show clinical response in these patients (Darouiche, 1998).

Several studies world wide demonstrate the emergence of non-*albicans* *Candida* as causative organisms in OPC (Sanchez-

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Vargas *et al.*, 2005; Badiee *et al.*, 2010), however there is a dearth of Microbiological studies on Candida colonization in HIV/AIDS patients from India. Further, the detection of Candida species which are either intrinsically resistant to fluconazole or can rapidly develop resistance like *C. glabrata* could adversely impact the utility of fluconazole as empiric treatment for Candidiasis in HIV/AIDS patients. Use of antifungal agents, especially the azole class, is widespread for the treatment of mucosal candidiasis. However, long-term use may lead to colonization with less susceptible Candida species or to the development of resistance among usually susceptible *C. albicans* strains and, ultimately, to refractory candidiasis.

This study was undertaken to investigate the changing epidemiology of Candida colonization in HIV/AIDS patients on Anti Retroviral Therapy (ART), examine its co-relation with CD4+ cell counts, study if there is any association between Candida speciation and CD4+ counts. This was compared with Candida colonization in HIV negative normal individuals.

MATERIALS AND METHODS

This prospective cross-sectional study was conducted over a period of 3 months between February 2014 and April 2014 at The Department of Microbiology, Bowring and Lady Curzon Hospital, Bangalore, Karnataka which is a tertiary care research and referral hospital attached to Bangalore Medical College and Research Institute. Patients attending the ART centre and the Integrated Counseling and Testing Centre (ICTC) at our hospital were recruited for the study. The study was undertaken after approval from the institutional ethics committee and National AIDS control Organisation (NACO) All participants provided informed consent.

A total of 500 HIV positive patients between the age group of 18 to 80 years, with positive confirmation of HIV-seropositive status, registered at the ART centre at our hospital and undergoing antiretroviral therapy and 100 HIV seronegative controls from ICTC within the same age group, with confirmation of HIV seronegative status with no clinical manifestations of oropharyngeal candidiasis as determined by a clinician through an oral examination and no other co-morbid conditions such as Diabetes, Hypertension etc were recruited after obtaining informed consent. Blood samples were obtained from HIV seropositive patients for estimation of CD4+ cell count by flow cytometry.

Collection of Oral Rinse Samples

Oral rinse samples were obtained from 500 HIV positive patients as part of their bi-annual CD4 count check, and from 100 HIV negative individuals who visited the ICTC for HIV testing.

Clinical samples were collected by rinsing the mouth with 10 ml of sterile phosphate-buffered saline (PBS; 0.1 M; pH 7.2), after holding in the mouth for 1 min prior to collection in a sterile container (Samaranayake *et al.*, 1986; White *et al.*, 2004).

Processing of samples and Culture

Each rinse was centrifuged (2,000 × g; 10 min), the supernatant was removed, and the deposit was resuspended in 1 ml of PBS. A portion (50 µl) of the concentrate was inoculated onto Sabouraud dextrose agar. Plates were incubated for 48 h at 37°C. Colonies suspected to be Candida based on Culture characteristics - Colony color, shape, texture, were sub-cultured onto CHRO Magar *Candida* (HiMedia, Mumbai, India) and incubated for 48 h at 37°C.

Identification methods

Candida was identified by conventional tests and all yeast isolates observed on CHROMagar were identified by colony morphology and pigmentation according to the manufacturer's instructions and as described by Odds and Bernaerts (Odds and Bernaerts, 1994). Isolates were further subjected to identification by gram staining, germ tube assay and sugar assimilation tests (Koneman *et al.*, 1992; Rippon, 1988).

RESULTS

Of the 500 HIV seropositive patients, 275(55%) were male and 225(45%) were female and of the 100 HIV seronegative, 51 were female (51%) and 49 were male (49%). The average age was 37±10 years and average years since diagnosis for HIV seropositive patients was 4±3 years with patients receiving ART from the same period of time. The CD4 +cell count ranged from 3 to 1587 cells/mm³. Of the 500 HIV seropositive patients, 70(14%) had CD4+ counts below 200/mm³ (mean, 117.6±54.9cells/mm³), 223(44.6%) had CD4+ counts between 200-499/mm³ (mean, 364.5 ± 76.9cells/mm³) and 207(41.4%) had CD4+ counts over 500/mm³ (mean, 757.9 ± 235.4cells/mm³). In the study group of 500, 166(33.3%) had Candida colonization and were culture positive and in 100 healthy controls 25 (25%) of these individuals were culture positive. Of the 70 patients with CD4 count <200, 39 (55.7%) had growth of which 33 (84.6%) were *Candida albicans*, *Candida crusei* 1(0.2%), *Candida lusitaniae* 1 (0.2%), *Candida parapsilosis* 1 (0.2%), *Candida tropicalis* 1 (0.2%), *Candida guilliermondii* 2 (0.5%).

In patients with CD4 count 200 – 499, 75 (33.6%) had growth of which *Candida albicans* 63 (84%), *Candida crusei* 2(2.6%), *Candida parapsilosis* 3 (4%), *Candida tropicalis* 2 (2.6%), *Candida glabrata* 1 (1.3%) *Candida guilliermondii* 4 (5.3%). In the group > 500, 52 (25.1%) had growth of which *Candida albicans* 43 (82.6%), *Candida crusei* 4(7.6%), *Candida lusitaniae* 1 (1.9%), *Candida parapsilosis* 1 (1.9%), *Candida tropicalis* 2 (3.8%), *Candida guilliermondii* 1 (1.9%). In 100 sero negative individuals 25 (25%) had growth of which 19(77%) *Candida albicans*, *Candida crusei* 1(3%), *Candida lusitaniae* 2(8%), *Candida parapsilosis* 2 (8%), *Candida tropicalis* 1 (3%) F

Table 1.

	CD4<200	200<CD4<499	CD4>500
GROWTH PRESENT	39	75	52
GROWTH ABSENT	31	148	155
	70	223	207

Table 2.

Species Name	Color	Characteristics
<i>Candida albicans</i>	White	...
<i>Candida krusei</i>	Red	...
<i>Candida lusitanae</i>	White	...
<i>Candida guilliermondii</i>	White	...
<i>Candida tropicalis</i>	White	...
<i>Candida glabrata</i>	White	...
<i>Candida lusitanae</i>	White	...

mouth, oesophagus, trachea, bronchi, and lungs as "major" opportunistic infections and important indicator diseases. Retrospective studies have shown that at least 58 to 81% of all AIDS patients contract a fungal infection at some time, and 10 to 20% die as a direct consequence (Scully *et al.*, 1994)

In the study group of 500, 166(33.3%) had CC and were culture positive. Oral Candida colonization in HIV-positive asymptomatic patients is known to be higher than in patients in other risk groups such as diabetes mellitus or other systemic disease (Vargas and Joly, 2002). The prevalence of CC (33%) in HIV-positive patients in the present study was lower compared to that in other studies (62.6% to 81%) but similar to study done by Maurya *et al.* (2013).

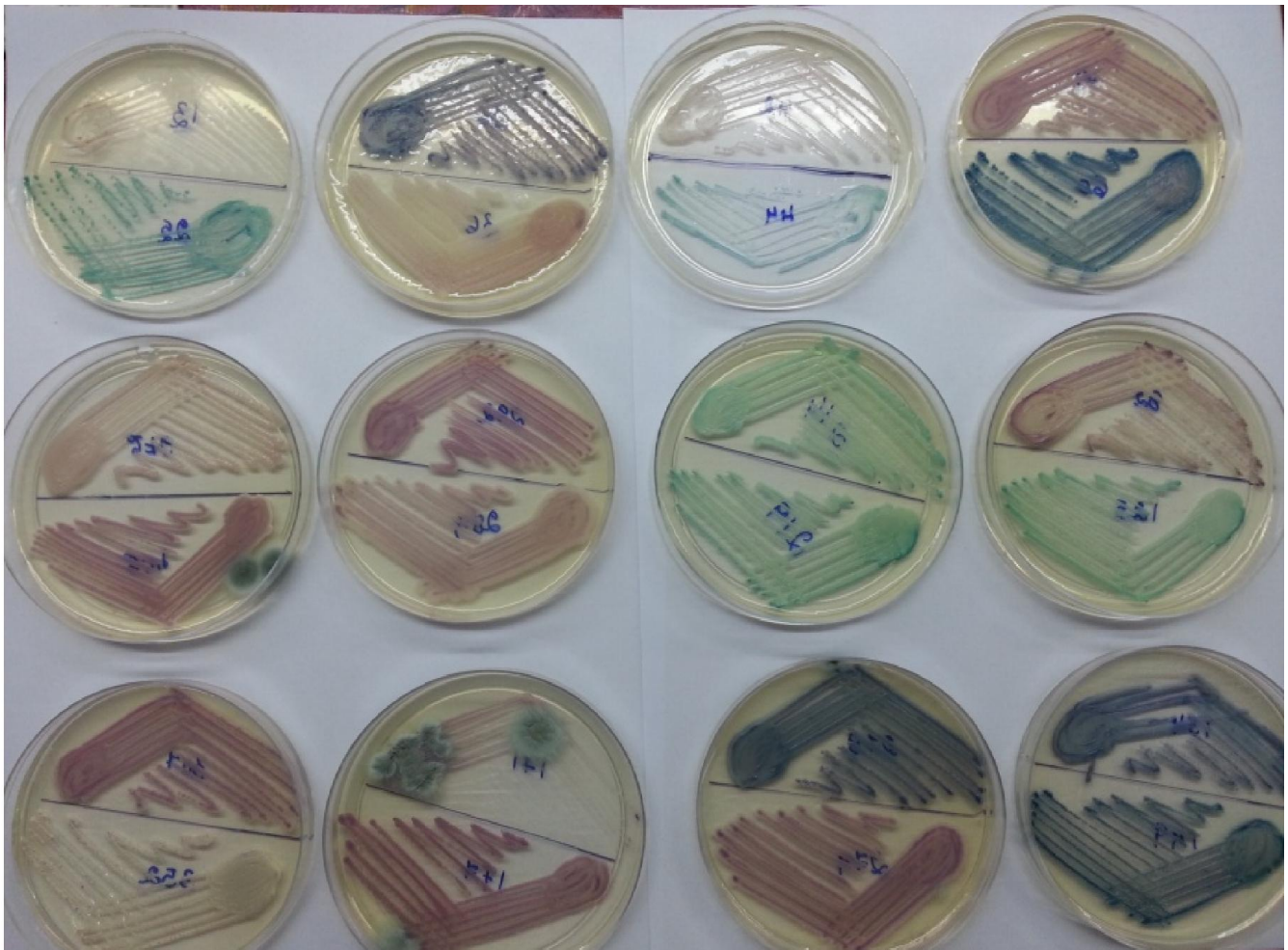


Fig. 1. Isolates on chrome agar

DISCUSSION

Asymptomatic carriage of *candida* species in the oral cavity is found irrespective of the immune status of individuals. Candidal colonization (CC) was defined as the presence of organisms at a mucosal site isolated by culture. CC rate can be affected by several factors such as hospitalization, abnormal nutrition, and smoking (Scully *et al.*, 1994). Candida infections, with oral thrush and esophagitis as frequent clinical manifestations, are the most common opportunistic infections encountered in AIDS. Ever since the first clinical definition of AIDS, the CDC/WHO have recognized candidiasis of the

The reason for the low isolation rate in the present study could be explained by single sampling performed in this study as compared to multiple sampling in other investigations.

Sero positive patients with CD4 count <200 had the highest number of candida colonization 39/70(56%) culture positive cases when compared to patients with CD4 count >200 to 499 75/223(34%) and CD4 count >500 52/207(25%). This shows there is significant correlation (P.0.0001) between CD4 cell count and CC. This has been also been reported by Schoofs *et al.* who had significant relationship between CC and

CD4+cell counts less than 200 cell/mm³ (Vijeta Maurya et al., 2013). But in contrast to this study, a study done by Costa et al. (2006) showed there was no association between CD4+ cell counts and candida colonization and they did not find a significant correlation between CC and CD4+ cell counts, as 16.25%, 61.2% and 22.6% of the patients in theirs had CD4+ cell counts ≤ 200, 201 to 500 and more than 500 respectively (Costa et al., 2006). Thus, in addition to the CD4+ count, some other factors appear to have significant effects on oral colonization by *Candida*.

C. albicans (84%) was the most frequent species isolated in our study, and 27 (16%) of isolates were non-*albicans* *Candida*. This is similar to a study done by Schuman et al. where *C. albicans* was most common isolate (87%) and non *C. albicans* were 13%. (Baradkar and Kumar, 2009) A study conducted in Italy by Barchiesi et al. who described increased frequency of isolation of non-*albicans* *Candida* species from 3% to 4% of isolates in 1988/1989 to 16% to 18% of isolates in 1990/1991 (Barchiesi et al., 1993). Similarly, in another Italian study, Morace et al. (1990) found that 25% of the yeast species isolated from persons with AIDS were non-*albicans* *Candida* (Morace et al., 1990). In Spain, Masia et al. evaluated 153 HIV-positive patients and found that 21% of these patients had non-*albicans* *Candida*, the most common being *C. glabrata* (MasiáCanuto et al., 1999). Our study shows that in India still, *C. albicans* continues to be the predominant colonizer than non-*albicans* *Candida*, and *C. guilliermondi* is emerging as a commonest non *albicans* colonizer. There was no association between candida species and CD4 counts. *C. albicans* is truly an opportunistic organism. It is the most common cause of candidiasis because it is the most thriving fungus at colonizing the oral cavity and so is in a position to take advantage of immune suppression in the host. *C. albicans* is the most adherent *Candida* species, which is probably due to its ability to adhere to many different ligands. It possesses other virulence factors, such as the secretion of hydrolytic enzymes and the ability to evade the immune mechanism (Cannon et al., 1995).

In the present study all the patients were on ART treatment and some of them had received fluconazole treatment. ART has been associated with dramatic decreases in the rate of HIV-related opportunistic infections. Our study suggests that ART does not influence oral CC, but it significantly prevented the development of OPC similar to study by Maurya et al. (2013). Treatment of HIV patients with prolonged courses of azole antifungal agents appears to have led to the development of azole-resistant candida strains, mostly the non *albicans* group. It is therefore necessary that the HIV- infected asymptomatic cases with CD4+ cell counts ≤ 200 cell/mm³ should be screened for oral candidiasis and species identified before starting treatment.

Our study had limitations that we could not do the antifungal susceptibility testing at that point of time due to logistics issues and also single sampling was performed. Even in Normal individuals we found 25% colonization with 77% *C.albicans* and 9% *C.parapsilosis* 8% *C.lusitaniae* 3% *C.lusitaniae* and 3% *C.krusei* this could be associated with cigarette smoking, alcohol usage, oral hygiene etc. Symptom-free oral carriage of *Candida* organisms has been recognized for many years.

Figures obtained on the frequency of yeast carriage in the oral cavity are dependent on isolation techniques and time of sampling. The reported prevalence in clinically normal mouths of healthy adults ranges from 3 to 48% and in 45 to 65% of healthy children (Scully et al., 1994).

Summary

Oral colonization of candida was associated with evolving immunodeficiency. Oral yeast colonization remain common in patients with HIV/AIDS, even with ART. *C. albicans* is the most common species, suggesting endogenous infection. CC was prevalent both in sero positives and negatives but more in sero positives. Emergence of non *albicans* candida was also seen in both the groups. These results highlight the need for screening for colonizers and also the need to increase awareness in healthcare providers and policy makers regarding emergence of non *albicans* species for appropriate therapeutic guidelines.

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