

Oral *Candida* isolates colonizing in human immunodeficiency virus infected patients during highly active antiretroviral therapy

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Abstract: Over 33 million people are diseased with HIV globally. Opportunistic infections continue to cause significant morbidity and mortality in patients with HIV infection. Oropharyngeal *Candida* infection is the most common opportunistic disease in HIV infected individuals. The advent of highly active anti-retroviral therapy (HAART) has reduced the prevalence of these opportunistic infections including candidiasis. The introduction of HAART has permitted suppression of viral replication and a partial recovery of CD4 T-lymphocyte count in HIV infected patients. This was a cross sectional prospective study including 100 HIV positive patients receiving HAART therapy in our hospital. Oropharyngeal specimens were collected and processed as per standard mycological methods. Out of the 100 patients included in this study *Candida* was isolated in 24 patients. Non *albicans Candida* was the most frequently isolated species accounting for 79.16% and the remaining were *Candida albicans* accounting for the 20.83% out of 24 smear positive samples. There was a low carriage of *Candida* in the oral cavity of patients with HIV and a higher prevalence of *C. albicans*, which may be the proper result of access to HAART for AIDS treatment. The use of accurate and reliable diagnostic methods which readily identify the non-*albicans* species could assist the clinicians in making the right therapeutic choices.

Keywords: Human immunodeficiency virus, *Candida*, HAART, Prevalence.

INTRODUCTION

Over 33 million people are diseased with Human Immunodeficiency Virus (HIV) globally and in India 2.31 million live with *acquired immunodeficiency syndrome* (AIDS) [1]. Opportunistic infections continue to cause significant morbidity and mortality in patients with HIV infection throughout the world[2]. Oropharyngeal *Candida* infection (OPC) is the most common opportunistic disease in HIV infected individuals, occurring in up to 90% of patients during the course of their disease. The advent of highly active anti-retroviral therapy (HAART) has reduced the prevalence of these opportunistic infections including candidiasis. The introduction of HAART has permitted suppression of viral replication and a partial recovery of CD4 T-lymphocyte count in HIV infected patients. A higher prevalence of oral *C. albicans* colonization may be a predisposing factor for the subsequent development of clinical thrush[2]. Although the incidence and prevalence of opportunistic infections have been reduced worldwide due to use of HAART,

OPC remains the most frequent HIV-associated oral lesion in most developing countries including India[3].

HIV infected patients tend to experience at least one episode of oropharyngeal candidiasis during the course of their illness. Although usually not associated with severe morbidity, oropharyngeal candidiasis can be clinically significant. Severe oropharyngeal candidiasis can interfere with the administration of medication, general health and quality of life of these patients[4]. Earlier, it was considered that *C. albicans* was the most common species causing oral candidiasis, but the emergence of HIV and AIDS, and the wide-spread use of azoles as prophylaxis and for treatment, however, lead to the increasing recovery of many other non-*albicans Candida* causing mucosal infections. Emergence of these non-*albicans Candida* species has led to a significant increase in drug resistance as they are increasingly found to not respond to conventional antifungal therapy[5]. This study determines the prevalence of oral *Candida* species in

HIV-infected patients receiving HAART of different age and sex.

MATERIALS AND METHODS:

A cross sectional prospective study was undertaken over period of period of two months under short term project by *Indian Council of Medical Research (ICMR)*. This study was carried out at the department of microbiology, Gajra Raja Medical College, Gwalior, Madhya Pradesh and was approved by the institutional ethical committee. Study group included 100 HIV positive patients confirmed by three rapid tests based on different principal as per NACO guidelines receiving HAART therapy. Informed consent was taken from all the patients before including them in the study. Patients of all age groups were included.

Two oral swabs were taken of each patient from oral lesions when present, the buccal mucosa, floor of mouth and from the dorsum of tongue under aseptic precautions and transported immediately to the laboratory. One swab was subjected to KOH wet mount preparation and gram's staining. The second swab was inoculated on Sabourauds dextrose agar (SDA)

supplemented with Chloramphenicol and incubated at 37°C for 24-72 hours. Isolates were identified by colony morphology on SDA plates[7]. Growth appears in 2 to 3 days as creamy, white pasty colonies. From the culture Gram's stain done to note the microscopic morphology. Isolates were differentiated into two groups with the help of germ tube test as follows[8]:

Germ Tube Test (Reynolds-Braude phenomenon)

A small portion of an isolated colony of the yeast to be tested was inoculated into the 0.5ml human serum and incubated at 37°C for two hours. After two hours of incubation, a drop of the yeast serum suspension was placed on a glass slide, overlaid with a cover slip and examined microscopically for the presence of germ tube under low power microscope. Test said to be positive, if tube like extension from the parent cell half the width and three to four times the length and no constriction at the point of attachment to yeast cell is seen in >30% of total yeast cells within 2hrs of inoculation, the isolate was considered presumptively as *Candida albicans*.

RESULTS

Table 1: Age-wise distribution

Age	Male		Female		Transgender	Total
	Number	Percentage	Number	Percentage	Number	
1-10	3	3%	0	0%	0	3
11-20	1	1%	0	0%	0	1
21-30	15	15%	9	9%	0	24
31-40	26	26%	16	16%	0	42
41-50	13	13%	8	8%	1	22
51-60	3	3%	2	2%	0	5
61-70	3	3%	0	0	0	3
Total	64	63%	35	35%	1	100

Mean age of the study is 37.09. The above table shows study group consists of 100 HIV seropositive

patients. 41% of patients are between 31-40 years. Mean age of study subjects is 37.09 years.

Table 2: Sex wise distribution

Gender	Number	Percentage
Male	64	64 %
Female	35	35 %
Transgender	1	1%
Total	100	100 %

The above table shows study group predominantly constituted of males at 64%, females constituted 35% and remaining 1% by transgender. Out of the 100 patients included in this study *Candida* was

isolated in 24 patients. KOH wet mount preparation was positive in 20 samples tested while gram's stain was positive in all 24 samples.

Table 3: KOH wet mount and Gram's stain positive Candida growing on SDA

Method	Number	Percentage
KOH wet mount positive	20	83%
Gram's stain positive	24	100%
Culture positive	24	100%

All smear positive budding yeasts cells with or without pseudohyphae were able to show growth on SDA.

Table 4: Distribution of *C. albicans* and Non albicans Candida

Type of Candida	Number	Percentage
<i>C. albicans</i>	5	20.83%
Non <i>albicans</i> Candida	19	79.16%
Total	24	100%

Non *albicans* *Candida* was the most frequently isolated species accounting for 79.16% and the remaining were *Candida albicans* accounting for the 20.83% out of 24 smear positive samples.

DISCUSSION:

Asymptomatic carriage of *Candida* species is a common finding in HIV positive patients. In our study we identify the risk of the most common opportunistic infection in HIV patients i.e. *Candida* species infection in the group receiving HAART. Asymptomatic carriage of *Candida* species in the oral cavity is found irrespective of the immune status of individuals. Many studies have been conducted on oral *Candida* colonization in healthy and immunocompromised individuals[9].

In the present study majority of the patients belong to age group 31-40 years with mean age of 37.09 years. These findings are almost similar to a study done by Vargas KG *et al.* [2]. In the present study, males belonged to a wider age spectrum and the females were considerably of middle-age population. There was a male preponderance accounting for 64% in present study. Vaishali Wabale *et al.*, [11] Vargas KG[2] and VP Baradkar *et al.*, [12] also reported similar results in their studies.

In present study, the growth of *Candida* was obtained from all samples 100% which is similar to other studies by Schmidt AM *et al.*[13] and Shobha ND *et al.*¹⁴ This indicates that SDA can be effectively used for isolation of *Candida* species. Oral carriage of *Candida* species is relatively common in individuals with HIV, affecting approximately 62% to 67% of them[10,15]. Our present study, there are 24 % of the samples collected from the oral cavities of patients with HIV which grew *Candida* colonies. Of these, 20.83% and 79.16% were *C. albicans* and Non *albicans*

Candida respectively, which was more frequent than the reported prevalence in the literature by other authors (32% to 62%)[16,17]. Our results suggest a low prevalence of oral cavity colonization by *Candida albicans* and as well as by non-*albicans* *Candida* species. Due to differences in the sample collection techniques used, time and frequency of sampling, yeast assessment methods, and the study population, results from the studies are not comparable. In addition, *Candida* colonization rate can be affected by several factors such as hospitalization, abnormal nutrition, and smoking[18].

CONCLUSIONS:

The proportion of *Candida* infections caused by *C. albicans* in HIV seropositive individuals has fallen and there is a shift in the distribution of *Candida* species in HIV positive patients towards the non-*albicans* *Candida* species. The use of accurate and reliable diagnostic methods which readily identify the non-*albicans* species could assist the clinicians in making the right therapeutic choices.

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