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Original article

Oral *Candida* species in healthy and HIV-infected subjects in Chennai, South India.

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Abstract

Objective: Candidiasis is the most common fungal infection in human immunodeficiency virus (HIV) - infected individuals. As there is sparse data on the oral *Candida* species in HIV- infected individuals in India, we characterized *Candida* species from the oral cavity in two cohorts - with and without HIV infection and with presence or absence of clinical oral candidiasis, in Chennai, South India.

Methods: Saliva samples were collected from 147 consecutive study participants by the oral rinse technique. *Candidal* species were isolated by culturing specimens on Sabouraud's dextrose agar. The pure cultures so derived were speciated using the commercially available ID32C system, and the results were interpreted using APILAB plus software.

Results: In the HIV seropositive group, the most commonly isolated candida species was *C.albicans* (86%) followed by *C.tropicalis* (23%), *C.guilliermondi* (6%), *C.krusei* (5%) and others (4%). In the healthy cohort without clinical candidiasis, C.tropicalis was the most commonly isolated species.

Conclusion: There appears to be a marked variation in oral *Candida* species found in HIV-seropositive and seronegative individuals in India. To our knowledge, this is the first attempt to identify oral Candida species in a South Indian population.

Key words: Oropharyngeal candidiasis, HIV, fungal infection

INTRODUCTION

HIV infection is a major global health problem. National AIDS Control Organization estimates the number of HIV- infected individuals in India at the end of 2006 to be 5.2 million [9]. About 90% of HIV- infected individuals suffer from at least one episode of oropharyngeal candidiasis before the widespread use of ART, during the course of their disease [31]. A dimorpic fungus, *Candida albicans* is the most commonly isolated species from oropharyngeal candidiasis in HIV- infected individuals [16]. The frequency of isolation of *C.albicans* and the clinical recurrence of oral candidiasis increases with the progression of HIV infection [23]. Repeated use of antifungals, though effective in the treatment of oral candidiasis, may lead to the development of resistance [28]. Certain *Candida (C)* species, namely - *C.glabrata* and *C.krusei*, have developed intrinsic resistance to fluconazole, a commonly used antifungal agent [25, 29]. Given the fact that antiretroviral treatment is not routinely available to HIV- seropositive individuals in South India, *Candida* continues to be the most common opportunistic infection. Our earlier study described the oral lesions in 300 HIV- positive symptomatic patients, 33% of whom had pseudomembranous candidiasis

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[17].

The primary objective of this study was to characterize oral *Candida* species in HIV- infected patients and to compare the results with those of a HIV- seronegative population in Chennai, South India. The secondary goal was to determine the Colony Forming Unit (CFU) of oral *Candida* in the two cohorts, because a positive correlation between CFUs and clinical candidiasis has been reported previously [30].

SUBJECTS AND METHODS

Study population:

A total of 147 subjects were included in the study.

Description of the study groups:

The patients were drawn from consecutive cases referred to Ragas Dental College and Hospital for oral/dental management over a period of six months from May 2005 to October 2005.

The symptomatic group consisted of patients exhibiting clinical oral candidiasis, while the asymptomatic group consisted of subjects without clinical oral candidiasis.

The patients were subdivided into 4 groups:

1) Asymptomatic HIV seropositive (AHP) (n = 52)
2) Symptomatic HIV seropositive (SHP) (n = 42)
3) Asymptomatic HIV seronegative (AHN) (n = 16)
4) Symptomatic HIV seronegative (SHN) (n = 37).

Seropositive group

Inclusion Criteria:

The seropositive status of the AHP and SHP group was confirmed by ELISA and Western blot. These patients were not on antifungals, antibiotics, corticosteroids or antiretrovirals for at least 30 days prior to inclusion in the study. EC-Clearinghouse 1993 criteria were used for definitive diagnosis of the clinical candidal infection [6].

Seronegative Group:

Inclusion Criteria:

The symptomatic group consisted mostly of patients who were either partial or complete denture wearers. The duration of symptomatic oral candidiasis varied from 3 months to a year. The patients included in this group were those who presented for routine dental treatment and were presumed to be HIV- seronegative on the basis of adequate exposure history and who were not being treated for any other chronic systemic disorders such as diabetes or hypertension [17].

Exclusion Criteria:

Patients who were on antibiotics, antifungals and corticosteroids were excluded from the study.

Ethical approval was obtained from the Institutional Review Board. Each patient's consent was obtained before recruitment into the study.

Culture and identification:

All subjects were requested to rinse their mouth for 60 seconds with 10ml sterile phosphate buffer saline (pH7.2, 0.1M). Each subject returned the rinse to a sterile container. The rinse was immediately centrifuged and concentrated by spinning at 1700 rpm for 10 minutes. The supernatant was discarded and the pellet resuspended in 2ml of sterile PBS. 100µl of sample were inoculated onto Sabouraud's dextrose agar plates (SDA), supplemented with Chloramphenicol (10 mg/ml) and incubated for 48 hours at 37℃ [24]. Pure culture of yeast was harvested and stored in vials containing sterile distilled water at -70℃ [23]. Colony forming units (CFU) were counted manually utilizing 0.001µl loop which was used to inoculate the SDA plate and the number of colonies formed was multiplied by 1000 which was represented as CFU/ml [2].

The organisms were speciated using the APILAB® commercially available kits, which identifies organisms based on sugar assimilation (BioMérieux, Marcy I'Etoile, France). Fresh cultures were made from the stored yeast samples and incubated for 24 hrs at 37℃ in SDA plates. They were then inoculated on the ID32C strips according to the manufacturer's instructions. The strips were incubated for 48 hr at 30℃ and read automatically using APILAB plus software (BioMérieux, Marcy I'Etoile, France) for speciation.

STATISTICAL ANALYSIS

Data entry, database management and analysis were done using SPSS version 10.0.5. Chi-square test was applied to determine statistically significant differences between the study groups (SHP, AHP, SHN and AHN) correlating age, gender, and *candida* species. ANOVA test was done to compare the mean CFU count among the study groups. Bonferroni test of multiple comparisons was also done to determine significant differences between the study groups. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 147 subjects were included in the study.

In the SHP group, 70% (29/42) were males and 31.0% (13/42) females. In the AHP group, 54% (28/52) were males and 46% (24/52) females. In the SHN group, 92% (34/37) were males and 8% (3/37) females. In the AHN group, 50% (8/16) were males and 50% (8/16) females. There was no statistically significant difference in the species distribution between genders.

Table I: Clinical variants of Candidiasis in the symptomatic groups

Clinical candidal lesion	SHP (n=42)	SHN (n=37)
Pseudomembranous candidiasis	24	-
Erythematous candidiasis	6	-
PC + EC	7	37
HC	3	-
PC + HC	2	-

SHP Symptomatic HIV positive; SHN Symptomatic HIV negative; EC Erythematous Candidiasis PC Pseudomembranous Candidiasis; HC Hyperplastic Candidiasis.

Table II: Prevalence of Candida species in the study groups

Candida species	SHP (n=42)		AHP (n=52)		SHN (n=37)		AHN (n=16)		p
	n	%	n	%	n	%	n	%	
C .albicans	36	86	35	67	34	92	2	13	0.00**
C .tropicalis	2	5	12	23	1	3	9	56	
C .krusei	2	5	0	0	0	0	1	6	
C .glabrata	0	0	0	0	1	3	4	25	
C .guilliermondi	1	2	3	6	0	0	0	0	
Others	1	2	2	4	1	2	0	0	

SHP Symptomatic HIV positive;AHP Asymptomatic HIV positive; Symptomatic HIV negative:AHN Asymptomatic HIV negative ; **p<0.01 Statistically significant

The age range of subjects in the AHP,SHP, AHN and SHN groups was 18-54 years, 25-52 years , 25-72 years and 25 -70 years respectively. On comparing the species distribution by age in the study groups, there was no statistically significant difference in the prevalence of various *candida* species.

Of the 42 cases in the SHP group, 24 had pseudomembranous candidiasis, 6 had erythematous candidiasis, 7 had a combination of pseudomembranous and erythematous candidiasis, 3 had hyperplastic candidiasis and 2 had a combination of pseudomembranous and hyperplastic candidiasis. The SHN group had both pseudomembranous and erythematous candidiasis (Table I).

On speciation using ID32C, the prevalence of species (decreasing order) was as follows (Table II):

In the SHP group, *C.albicans, C.tropicalis, C.krusei, C. guilliermondi* and *Debaryo polymorphus*.

In the AHP group, *C.albicans, C.tropicalis, C.guilliermondi C.kefr and C.sake*.

In the SHN group, *C.albicans, C.tropicalis, C.glabrata* and were other species namely *Pichia etchellsi/ carsonii*.

In the AHN group, *C.albicans, C.tropicalis, C.krusei and C.glabrata*. There was a statistically significant difference in the percentage of all species (p=0.000) between the study groups (Table II).

Other organisms identified in our study were *C. lusitaniae, C. kefyr, C. sake, C. sphaerica, Zygosaccharo* spp., *Debaryo polymorphus* and *Pichia etchellsii/carsonii* comprising 3% of the total study population. *Debaryo polymorphus* and *Pichia etchellsii/carsonii* were isolated for the first time in HIV seropositive and seronegative groups respectively.

Table III: CFU distribution among the study groups

Study groups	Mean	SD	Median	Range	p value
SHP (n=42)	81.88	129.52	24.00	4.0-486.0	0.00**
AHP (n=52)	10.91	11.28	6.200	0.02-45.0	
SHN (n=37)	6.03	4.57	4.00	2.0-18.0	
AHN (n=16)	3.6	4.73	1.78	.01-18.0	

SHP Symptomatic HIV positive; AHP Asymptomatic HIV positive; SHN Symptomatic HIV negative AHN Asymptomatic HIV negative; **p<0.01 Statistically significant

Table IV: Mean difference in CFU distribution among study group pairs

Groups	Mean difference	Std.Error	p value
SHP Vs AHP	35.8453	8.63	0.00**
SHP Vs SHN	40.2962	8.69	0.00**
SHP Vs AHN	43.1844	26.41	0.631
AHP Vs SHN	4.4509	8.75	1.000
AHP Vs AHN	7.3391	26.43	1.000
SHN Vs AHN	2.8882	26.45	1.000

SHP Symptomatic HIV positive; AHP Asymptomatic HIV positive; SHN Symptomatic HIV negative; AHN Asymptomatic HIV negative; **p value<0.01 Statistically significant

Mean CFU was 81.88 x 10^3/ml (±129.52) in the SHP group, 10.91 x 10^3/ml (±11.28) in the AHP group, 6.03 x 10^3/ml (±4.57) in the SHN group and 3.6 x 10^3/ml (±4.73) in the AHN group. CFU was higher in the SHP group than in the AHP, SHN, or AHN groups, and the difference was statistically significant (p=0.000). The median difference in CFU in the SHP, AHP, SHN, and AHN group was 24 x 10^3/ml, 6.20 x 10^3/ml, 4 x 10^3/ml and 1.78 x 10^3/ml respectively (Table III).

A statistically significant difference (p<0.01) was observed only for the mean difference in CFU between the SHP vs AHP groups (35.84 x10^3/ml) and the SHP vs SHN groups (40.29 x10^3 /ml)(Table IV). There was no statistically significant difference between the other groups. Smokers represented 25% of the HIV- seropositive group and 15% of the HIV- seronegative group. These patients smoked only occasionally. A statistically significant relationship could not be established between smoking habit and CFUs. None of the subjects had the habit of alcohol consumption.

DISCUSSION

Oral candidiasis is the most common oral manifestation of HIV- seropositive patients. In India, it has been reported in 21 to 85 % of HIV- seropositive individuals [1, 17, 18]. Similar prevalence rates have been reported from other Southeast Asian countries [11, 12, 7].

In our study, the pseudomembranous variant of oral candidiasis was the most common lesion in SHP (57%), a finding similar to our earlier report [17] and to the prevalence of 52.5% observed in 101 Cambodian adults [19]. Of the 37 patients in the SHN group, 22 (60%) were denture wearers. In addition to exhibiting erythematous candidiasis, these patients also showed pseudomembranous candidiasis in the region of the soft palate. This is attributable to poor oral / denture hygiene practices and wearing dentures overnight [23, 25]. The remaining 15(40%) of 37 were not denture wearers. We have observed this pattern of pseudomembranous candidiasis and erythematous candidiasis (usually as central papillary atrophy on the dorsal surface of tongue) in patients, at our hospital, showing iron deficiency / vitamin B12 deficiency anemia (unpublished data).

C.albicans was the most commonly isolated candidal subtype in SHP (86%), AHP (67%) and SHN (91%). This finding is similar to reports from Thailand, Italy, USA and Mexico [30, 11, 12, 4, 26, 31].

The prevalence of *C.albicans* and non-*C.albicans* isolates was found to be 85% and 25% respectively in the HIV-seropositive group, a finding consistent with a Mexican study which reported *C.albicans* 84% and non-*C.albicans* 27% [26].

The second most common candidal subtype was *C. tropicalis*, which was seen more frequently in the AHN (56%) and AHP groups (23%) than in the SHP (5%) and SHN groups (3%). The third most common candida species was *C.guilliermondii*, which accounted for 2% of the SHP group and 6% of the AHP group. However, it was not isolated from the seronegative groups. *C.krusei* was isolated from the SHP (5%) and AHN (6%) but not from the AHP and SHN groups in our study. This prevalence rate is similar to an earlier Indian report in which *C.krusei* and *C.glabarata* prevalence was only 3% in 125 patients [8].

C. glabrata was isolated from neither the symptomatic nor asymptomatic HIV- seropositive individuals, while *C. tropicalis* was isolated from both subgroups. This was in contrast to the report from India in which only *C. glabrata* was isolated [8]. This may be attributable to factors such as geographic distribution and endogenous yeast flora. Other investigators have shown the oral prevalence of *C. krusei* and *C. parapsilosis*, and not *C. albicans*, to be high in specific population groups from different geographic locations in Asia [20, 32]. Our study group was from South India, while the earlier 2004 study by Latiff et al was from North India [8]. *C. glabrata* has also been reported by other investigators from the USA and Cambodia [27, 31]. Detailed research is needed to elucidate the variations and differences in candidal species isolated from geographically diverse cohorts and from normal subjects and those with pathological conditions.

Another factor that may contribute to the difference in prevalence of candida species and carriage rates is the different sample collection procedures utilized by different investigators, diurnal variation (carriage is higher in mornings and early afternoons) and the techniques used to identify candidal species.

We also observed that the overall density of oral candidal carriage as reflected by CFU/ml was higher in the HIV-seropositive group than in the HIV- negative group. The high CFU may be explained by the fact that the subjects in the SHP group were immunocompromised. These findings underline the fact that immunosuppression increases the candidal carriage in SHP patients. Interestingly, even in the absence of clinical candidiasis, the CFU was higher in the AHP group than in the SHN group, which is probably due to the alteration of the mucosal immune system in HIV patients [30,5].We did not find any correlation between CFU and other factors such as smoking in any of the groups.

Our data show that *C. albicans* is the most common isolate in our cohort of an HIV- infected population. However, the prevalence of non-*C. albicans* species was high in comparison with some previously reported studies from other regions of the world [22]. Curiously, *C. glabrata*, an important emerging drug resistant species, was not isolated from the HIV- infected population in the present study. Also, *C. tropicalis* was the most prevalent species in the asymptomatic HIV- negative subgroup. To our knowledge, this is the first study from a South Indian population wherein oral Candida species have been studied. The results of this baseline study should be considered with attention to the small sample size and the limitations of the APILAB® technique used. The present study shows that there is variation in candida species in HIV- seropositive and seronegative subjects. However, a larger cohort and utilization of molecular techniques which can identify a wider range of species including *C.dubliniensis* will further add to our knowledge of candidiasis, a major opportunistic infection in the HIV- infected population of India.

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