

# Comparison of Candidal Species in Oral and Cervical Smears in Human Immunodeficiency Virus Positive Women- A Prospective Study.

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## ABSTRACT

**Background:** Mucosal candidiasis which includes oropharyngeal and vaginal candidiasis, is suggested to be an early sign in immunocompromised individuals especially in HIV positive patients Aims and Objectives: To compare the frequencies of oropharyngeal and vaginal candidal colonization among HIV-seropositive women. **Methods:** We carried our study in 70 HIV positive women. A detailed medical history was taken. The variables recorded were age, educational status, antibiotics usage, occupation, marital status and usage of contraceptives. Oral and cervical smears were taken and cultured on Sabouraud dextrose agar and HiCrome agar, and candidal colonies were counted. Statistical analysis was performed with SPSS for Windows: IBM Corp. Version 20.0. Armonk, NY, USA. **Results:** Oropharyngeal candidal colonization was seen in 42 (60%) women. Candida albicans was most commonly identified (90%), followed by C tropicalis (5%) and C glabrata (3%). Vaginal colonization was identified in 38 (54%) women. C. albicans was most commonly identified (80%), followed by C. glabrata (15%) and C. tropicalis (3%). **Conclusion:** There were more non albicans species in vaginal smears than oral smears, suggesting dissimilar pathogenesis in both the sites.

**Keywords:** Candidiasis, Cervical smear, Diabetes, HIV, Sabouraud Dextrose Agar, HiChrome agar.

## INTRODUCTION

The most common fungal infections of mucosa are caused by Candidal species mainly Candida albicans particularly in immunocompromised persons.<sup>[1]</sup> The term mucosal candidiasis is given in cases of oropharyngeal, esophageal, and vaginal candidiasis, and is supposed to be the commonest opportunistic fungal infection in HIV patients.<sup>[2]</sup> Mucosal candidiasis shows varied occurrence, with oral and esophageal candidiasis seen mainly in women who are suffering with severe to profound immunocompromised status, whereas vaginal candidiasis occurs commonly not related to immunosuppression. Studies have shown that mucosal candidiasis, especially vaginal candidiasis was frequently recurrent and unresponsive to

conventional therapy in HIV-seropositive women and that new onset or greater than before occurrence of vaginal candidiasis might signify the initial expression of HIV infection.<sup>[2,3]</sup>

But the presence of mucosal candidiasis is seen in other conditions like use of antibiotics, corticosteroids etc regardless of HIV serostatus. Vaginal candidiasis is also seen in women using oral contraceptives, hormone replacement therapy and antibiotics.<sup>[4,5]</sup>

We carried out this study to compare the frequencies of oropharyngeal and vaginal candidal colonization among HIV-seropositive women.

## MATERIALS AND METHODS

We carried our study in 70 HIV positive women of different socio-economic backgrounds at Nireekshana ACET center, Narayanaguda, Hyderabad, Telangana State, India.

### Inclusion Criteria:

1. HIV positive women
2. Women who were cooperative for oral and cervical smears

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**Exclusion Criteria**

1. Uncooperative women
2. Women who were younger than 18 years were excluded

Ethical clearance was obtained from the institution and informed consent was taken from all the subjects. After explaining the details about the study, the participants were asked about their socio-demographics and a detailed medical history was taken. The variables recorded were age, educational status (uneducated or educated up to primary, secondary or tertiary school), HAART therapy, antibiotics use, occupation (unemployed or self-employed), marital status and the usage of contraceptives. Oral and gynaecologic examinations were carried out. Oral and cervical smears were taken and the collected specimen was cultured on Sabouraud dextrose agar (SDA) and HiCrome agar and colonies were counted.

Candidal colonization was defined as the presence of organisms at a mucosal site, as determined by culture on SDA incubated at 300C. HiCrome Candida Differential Agar is used in cases where there is need for quick isolation and identification of Candida species from mixed cultures, as it has chromogenic or fluorogenic hexosaminidase substrates. It is useful in differentiation of Candida species such as *C.albicans*, *C.krusei*, *C.tropicalis* and *C.glabrata* on the basis of their colour and colony morphology. The role of chloramphenicol in this medium is to suppress the associated bacterial flora. *C.albicans* appear as light green coloured smooth colonies, *C.tropicalis* appear as blue to metallic blue coloured raised colonies, *C.glabrata* colonies appear as cream to white smooth colonies, while *C. krusei* appear as purple fuzzy colonies.

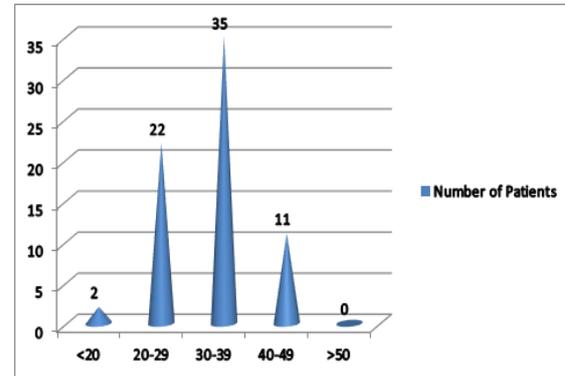
Statistical analysis was performed with SPSS for Windows: IBM Corp. Version 20.0. Armonk, NY, USA. Significance test was done with independent samples t-test. Results were displayed as number and percentages, and for all statistical tests, P < 0.05 was considered statistically significant. The associations of oropharyngeal and vaginal candidal colonization and candidiasis with HIV serostatus, CD4 lymphocyte count or category (<200, 200–499, and >500 cells/mm<sup>3</sup>), risk activities, and current medications were tabulated and subjected to statistical analysis.

**RESULTS**

When age of all the women was considered, we found that 35 (50%) of them were between 30 to 39 years of age, followed by 22 (31.43%) women between 20 to 29 years of age, with least number i.e. 2 (2.85%) women of less than 20 years age [Table 1 and Figure 1].

**Table 1: Number and Percentage of patients in various Age groups.**

Age (Years)	Number of Patients	Percentage of Patients
18-20	2	2.85
20-29	22	31.43
30-39	35	50.00
40-49	11	15.71
>50	0	0.00



**Graph 1: Number and Percentage of patients in various Age groups.**

The educational status of the women was recorded based on Kuppu Swami Scale. It uses education and occupation of the head of the family and monthly family income to calculate the socio-economic scale. We found that 38 (54.29%) women studied till secondary school, followed by 24 (34.29%) uneducated women and 7 (10%) studied till primary school, only one studied till tertiary school [Table 2].

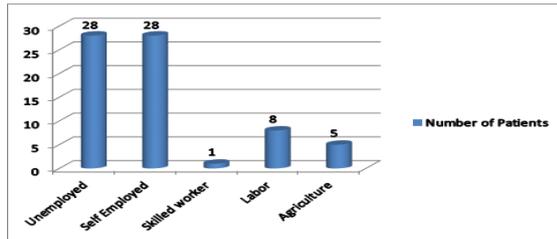
**Table 2: Categorization of Number and Percentage of patients according to their Education status.**

Education Status	Number of Patients	Percentage of Patients
Uneducated	24	34.29
Primary School	7	10.00
Secondary School	38	54.29
Tertiary School	1	1.43

Women were categorized based on their occupation. 28 (40%) of women were unemployed and self employed, 8 (11.43%) were labors and 5 (7.14%) were agriculture workers [Table 3 and Figure 2].

**Table 3: Categorization of Number and Percentage of patients according to their Occupation**

Occupation	Number of Patients	Percentage of Patients
Unemployed	28	40.00
Self Employed	28	40.00
Skilled worker	1	1.43
Labor	8	11.43
Agriculture	5	7.14



**Graph 2: Categorization of Number and Percentage of patients according to their Occupation**

Women were also categorized according to their marital status. 46 (65.71%) of them were widows and 24 (34.29%) were married [Table 4]. The difference being statistically highly significant (p=0.002).

**Table 4: Categorization of Number and Percentage of patients according to their Marital Status**

Marital Status	Number of Patients	Percentage of Patients	P value
Married	24	34.29	
Widow	46	65.71	

All the women were asked whether they were using any contraceptives and it was found that 46 (65.71%) of them were not using contraceptives, whereas 24 (34.29%) did used [Table 5]. The difference being statistically highly significant (p=0.002).

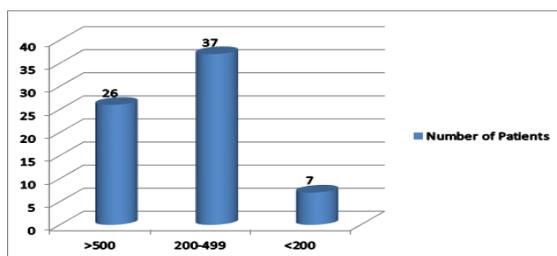
**Table 5: Number and Percentage of patients who are using contraceptives**

Contraceptive Use	Number of Patients	Percentage of Patients	P value
Yes	24	34.29	
No	46	65.71	

Few of them were diabetic and few had TB. All the women were taking medications for HIV therapy. When CD4 count was assessed, 37 (52.85%) had CD4 count between 200 to 499 per mm, whereas 26 (37.14%) had >500 per mm and 7 (10%) had <200 per mm [Table 6 and Figure 3].

**Table 6: Correlation of Number and Percentage of patients with CD4 count**

CD4+T Cell Count per mm	Number of Patients	Percentage of Patients
>500	26	37.14
200-499	37	52.85
<200	7	10.00



**Graph 3: Correlation of Number and Percentage of patients with CD4 count**

**Oropharyngeal Candidal colonization**

Culture results for oropharyngeal candidal colonization were available for all the 70 participants. We observed oropharyngeal candidal colonization in 42 (60%) of the HIV seropositive women. We also found that CD4 lymphocyte count in women with oropharyngeal candidal colonization was significantly lower than those without oropharyngeal colonization (314/mm<sup>3</sup> vs. 395/mm<sup>3</sup>, P =.004; significant). When other parameters were combined with candidal colonization, it was found that oropharyngeal candidal colonization was high among elder women and those using antibiotics.

After culture, Candida species identification was carried out. Candida albicans was most commonly identified (90%), followed by Candida tropicalis (5%) and Candida glabrata (3%) [Figure 1 and 2].



**Figure 1: Showing candidal colonies on SDA**



**Figure 2: Showing candidal colonies on HiChrome Agar**

**Vaginal Candidal Colonization**

Vaginal colonization was identified in 38 (54%) of women. CD4 lymphocyte count in women with vaginal candidal colonization was significantly lower than those without oropharyngeal

colonization (368/mm<sup>3</sup> vs. 384/mm<sup>3</sup>, P =.004: significant). When other parameters were combined with candidal colonization, it was found that vaginal candidal colonization was high among women using antibiotics, antihyperglycemic therapy and contraceptives.

Among the Candida species, C. albicans was most commonly identified (80%), followed by C. glabrata (15%) and C. tropicalis (3%). We observed more non albicans species in vaginal smears than oral smears.

**Table 7: Candidal species in Oral and Vaginal smears**

Candidal Species	Percentage In Oral Smears	Percentage In Cervical Smears
C Albicans	90	80
C. Tropicalis	5	3
C Glabrata	3	15

## DISCUSSION

Opportunistic infections like candidiasis are usually seen in diverse infections that bring about immunodeficiency like diabetes mellitus, neoplasia, organ transplantation and AIDS. The scale of Candida infection is varied, ranging from asymptomatic colonization to pathogenic forms. The main risk of candidiasis in HIV patients is supposed to be lowered CD4+ T-lymphocyte count, mainly if the count falls below 200 cells/mm<sup>3</sup>. Recently more and more non-albicans Candida species are isolated from oral and cervical smears of HIV patients and this is a matter of high concern as non albicans species is thought to be mainly due to advanced immunosuppression or resistant to antifungal therapy and are supposed to be invasive forms of Candidiasis.<sup>[1,5,6]</sup>

India had about 2.1 million people living with HIV at the end of 2016. This is one of the most studied dreadful disease. The primary objective of our study was to compare the frequencies of oropharyngeal and vaginal candidal colonization among HIV-seropositive women. We noticed that oropharyngeal colonization with Candida species was seen in 60 % of women. This increased finding might be due to the fact of immunosuppression seen in these women. The finding that increased prevalence of oropharyngeal colonization in women with lowered CD4 count might be due to further reduced immunue status in them. Our findings are in accordance with Phelan et al and Feigal et al. They also found similar results in HIV positive men.<sup>[7,8]</sup>

The species of Candida identified in oral and cervical smears in our study were albicans followed by tropicalis. This finding was similar to previous studies of Sangeorzan et al.<sup>[9]</sup>

We found more non C. albicans species in vaginal smears than oral smears. This is in accordance with Schuman et al.2 Spinillo et al also reported similar finding of non albicans species in vaginal candidiasis in HIV positive women, but the

percentage in their case was much higher than our study.<sup>[5]</sup>

We also found a higher incidence of candidiasis in oral smears when compared to cervical smears. This shows that pathogenesis of candidiasis might be different in oropharyngeal and vaginal candidiasis. This is similar to the findings of Schuman et al.<sup>[2]</sup> However Imam et al and Spinillo et al found higher frequency of vaginal candidis.<sup>[3,5]</sup>

A Women's Fungal Study was carried out by the Terry Beirn Community Program for Clinical Research on AIDS, a randomized clinical trial of weekly fluconazole vs. placebo for the prevention of mucosal candidiasis in women with late HIV infection. Here women were evaluated for occurrence of oropharyngeal and vaginal candidiasis by standardized diagnostic evaluations. They found that Oropharyngeal candidiasis occurred more often than vaginal candidiasis. Among the candidal species, C. albicans and C. glabrata were most frequent ones and also noted that antifungal usage was related with greater than before probability of colonization with a non albicans species.<sup>[10]</sup>

We found higher frequency of candidal colonization in women with fewer CD4 count. This is similar to previous studies. However Duerr et al reported a higher frequency of vaginal candidal colonization among HIV-seropositive women with > 200 CD4 lymphocyte counts.<sup>[11]</sup>

The varying results might be due to differences in study design, methodology, sample size, socioeconomic status, data analysis and definitions of vaginal candidiasis. Our findings of occurrence of mucosal candidiasis and the different species in HIV positive women will provide additional data on the relationship of mucosal candidiasis and progressive HIV infection. Centers for Disease Control and Prevention suggest that frequent oropharyngeal and vaginal candidal infections should prompt HIV testing and our study also supports this view.<sup>[12,13]</sup>

## CONCLUSION

We found an increased prevalence of mucosal candidal colonization of the oropharynx and vagina among HIV seropositive women. Oral candidiasis was seen more with progressive immunodeficiency, whereas vaginal candidiasis was not associated with immunocompromise. We found a higher than expected occurrence of non-albicans Candida species. Further such studies will enhance our knowledge about candidal colonization as an early marker in HIV patients and the role of different candidal species in HIV patients.

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