Original Article

Quantification of Oral Candidal Carriage Rate and Prevalence of Oral Candidal Species in HIV Patients with and Without Highly Active Antiretroviral Therapy

Nitesh Perla[1,2], Sourab Kumar[2], Abhishek Jadhav[2], Payoshnee Bhalinge[3], Mrunalini Dadpe[3], Siddharth Acharya[2]

[1]Oral Pathologist, Dental Clinician, [2]Department of Oral Pathology, D Y Patil School of Dentistry, Navi Mumbai, [3]Department of Oral Pathology, M. A. Rangoonwala College of Dental Science and Research Centre, Pune, Maharashtra, India

Abstract

Background: Most documents review decrease in the prevalence of HIV related oral lesions to the tune of 10-50% following the advent of HAART. However long term use of HAART on oral health status of HIV infected subjects is poorly documented. Also antifungal agents can effectively treat mucosal candidiasis. However their use can lead to colonization with less susceptible strains among normal susceptible strains. **Aims and Objectives:** To know the candidal carriage rate (i.e. presence/absence of candidal growth), candidal density(CFUs/ml) & species variations (species diversity) in HIV positive individuals with and without highly active anti-retroviral therapy (HAART), attending the regional voluntary Counselling and Confidential Testing Centre (VCCTC). **Materials and Methods:** The study population were categorized into 3 groups. Method followed were Germ tube test, Chlamydospore formation test, CHROM-Agar test. **Results:** Quantification comparison study of candidal carriage rate, density with detection of various candidal species in the oral cavity of HIV-positive individuals with and without HAART therapy was conducted. **Conclusion:** HIV positive individuals with HAART therapy treatment prooved higher candidal carriage rate and lower density than Non-HAART category.

Keywords: AIDS, candidiasis, carriage rate, HIV, Voluntary Counseling and Confidential Testing Centre

Introduction

Due to of the emergence of other and newer species of *Candida* as pathogens and a development of change in the susceptibility pattern of *Candida albicans*, it necessitates the isolation and identification of the causative species.[1-6]

The advent of highly active antiretroviral therapy (HAART) has changed the scenario of HIV infection and has become a standard treatment for HIV infection.[7] It induces a marked reduction in viral load and increase in $CD4^+$ cell count leading to decline in the morbidity and mortality of HIV-infected patients.[8,9] In HAART therapy, a range of different combination of drugs are used and each combination of drugs has advantages and disadvantages. They are administered simultaneously to bring about sustained block in viral replication and restore immune function as well as to minimize resistance to drugs.[8]

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Aims and objectives

1. To analyze the oral candidal carriage rates in HIV-positive individuals who are undergoing antiretroviral therapy and in patients who are not on antiretroviral therapy
2. To quantitatively assess the candidal density in the above-mentioned groups
3. To assess the strain diversity in the above mentioned groups.

Materials and Methods

The study population included 30 patients each of Group I and Group II attending the Voluntary Counseling and Confidential

Address for correspondence: Dr. Sourab Kumar, Department of Oral Pathology, D Y Patil School of Dentistry, Nerul, Navi Mumbai - 400 706, Maharashtra, India. E-mail: sourab.kumar@dypatil.edu

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Testing Centre (VCCTC), ART center.

1. Group I: Included 30 patients who were people living with HIV/AIDS naive to HAART
2. Group II: Included 30 patients who were people living with HIV/AIDS who were registered for HAART and started on drug regimen

   The patients from Group I and Group II were direct walk-in patients in the VCCTC, who were positive for HIV by three tests (COOMBS AIDS; TRI DOT; and TRI LINE), according to the guidelines of the National AIDS Control Organization.
3. Group III: Included 30 HIV seronegative healthy subjects as controls.

Inclusion criteria

1. 30 HIV-positive patients who were being treated with HAART regimen, at least for a duration of 1 month and with a known $CD4^+$ T-lymphocyte count
2. 30 HIV-positive patients who were not yet initiated with the treatment of HAART and with a known $CD4^+$ T-lymphocyte count.

Exclusion criteria

1. Patients with a history of tuberculosis, diabetes mellitus, cardiovascular diseases, rheumatoid arthritis, and any systemic ailments were excluded. Pregnant females and denture wearers were also excluded from the study
2. HIV-positive patients with HAART duration less than a month were also excluded.

Methods

1. All the patients included in this study were asked to rinse with 10 ml of normal saline for 60 s before expectorating into the sterile container [Figure 1: Figure 2]
2. The oral rinse sample was immediately taken to the microbiology department for inoculation of the sample on a Sabouraud dextrose agar (SDA), specific for candidal growth
3. 0.1 ml of undiluted oral rinse sample was inoculated on two plates of SDA containing chloramphenicol
4. 0.1 ml of diluted (10^{-1}) oral rinse sample of HIV-positive patients was also inoculated on two plates of SDA plates containing chloramphenicol.

   Note: 10^{-1} dilution of the oral rinse sample is prepared by mixing 0.1 ml of oral rinse sample of HIV-positive patients with 0.9 ml of sterile normal saline.
5. The above plates were then incubated aerobically at 37°C for 48–72 h
6. The growth appeared in 2–3 days as creamy white, smooth, pasty colonies. In a few, the growth was observed within 24 h, i.e., overnight incubation
7. The complete growth of any candidal colonies on the culture plates was recorded as a positive growth and the subject as positive candidal carrier (i.e., positive candidal carriage rate)
8. The number of colonies on each plate was counted manually, and an average count of both the diluted (10^{-1}) plates was taken
9. The number of colony-forming units (CFU's) per ml was calculated to indicate the candidal density [Figures 3-6].

The calculation was as follows:

- N no of colonies in 0.1 ml of 10^{-1} dilution (since 0.1 ml of 10^{-1} was spread on the agar plate)
- 10N no of colonies in1 ml of 10^{-1} dilution of the normal saline
- 100N colonies in 1 ml of sterile saline which gives the CFU's/ml.

The representative colonies of *Candida* species on SDA plate were then purified on blood agar with a streak method. Further identification of species was done by VITEK test using the purified colonies grown on blood agar [Figures 7 and 8].

Some of the samples were tested retrospectively with the help of germ tube test, chlamydospore formation test, and with CHROM agar.

Germ tube test

The principle of this test is the ability of *C. albicans* and its variants to produce germ tubes when incubated with various

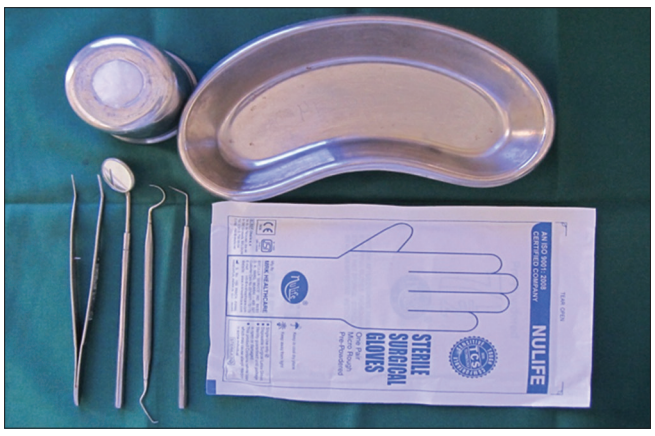


Figure 1: Armamentarium used for clinical examination of patients

Figure 2: Sterile bottle containing10ml of sterile normal saline for patients oral rinse sample

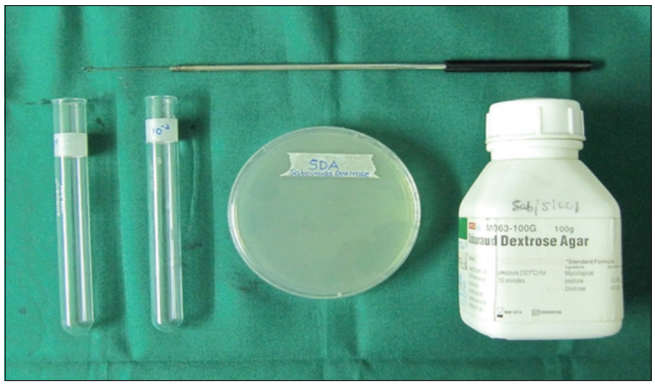


Figure 3: Armamentarium used for inoculation of oral rinse sample

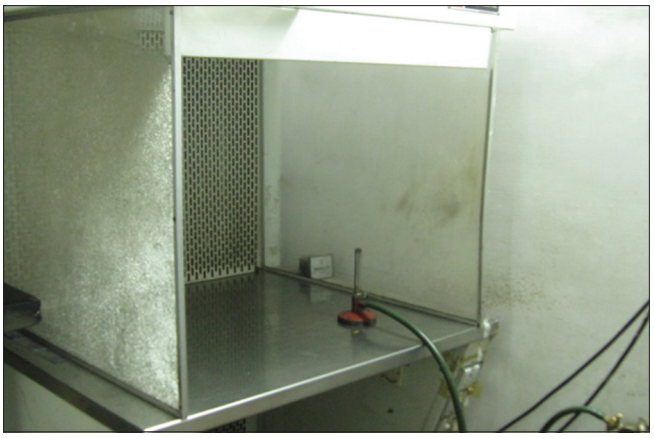


Figure 4: Working place for the aseptic inoculation of oral rinse sample

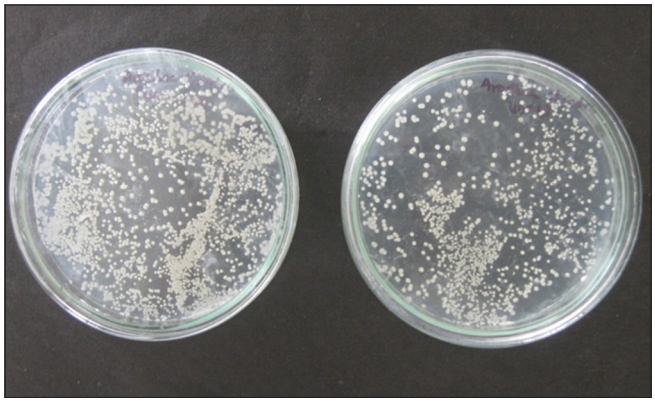


Figure 5: Positive growth of *Candida* species in undiluted and diluted (10^{-1}) plates

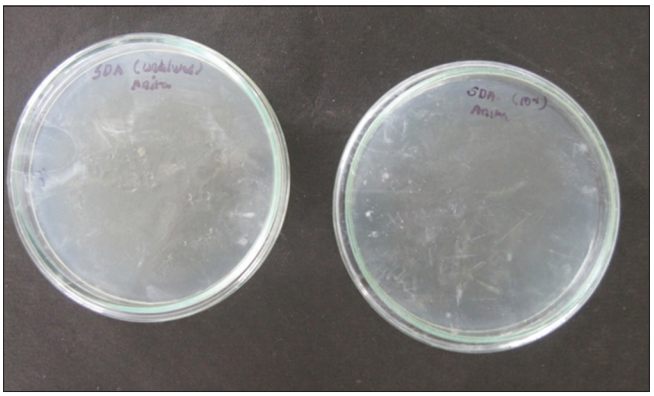


Figure 6: Negative growth of *Candida* species in undiluted and diluted (10^{-1}) plates

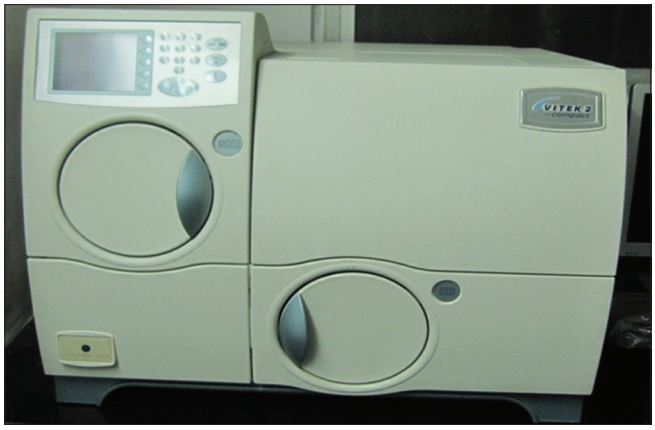


Figure 7: VITEK machine for rapid identification of different strains of *Candida* species

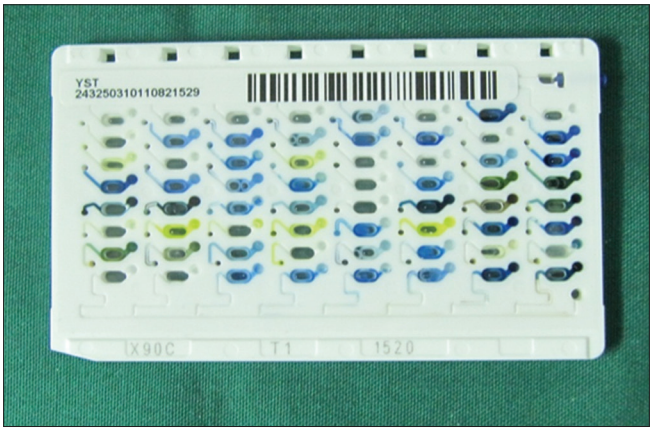


Figure 8: Yeast identification card after performing the test in VITEK machine

substances such as human or sheep serum, rabbit plasma, egg albumin, saliva, tissue culture medium, thioglycolate trypticase soya broth, and various peptone mediums. This is a rapid screening procedure for differentiating *C. albicans* from other *Candida* species.

Chlamydospore formation test

Cultivation on cornmeal agar facilitates and appreciates chlamydospore formation. This property is peculiar to *C. albicans* and to very rare isolates of *Candida tropicalis* and *Candida stellatoidea*. The chlamydospore has been defined as a thick walled nondeciduous intercalary or asexual spore formed by rounding off of a cell or cells.

CHROMagar test

CHORMagar is a novel differential culture medium for isolation and presumptive to identification of different species

of *Candida* and has revealed mixtures of *Candida* species in many types of clinical samples more often than would have been expected. The species of *Candida* can be identified by different colored colonies. Different colored colonies produced on the CHROM agar are as follows:

1. *C. albicans*: light green; 2. *Candida glabrata*: purple 3; *C. tropicalis*: blue with pink hallow; 4. *Candida parapsilosis*: cream; 5. *Candida krusei*: pink (rough, fuzzy spreading) l; and 6. *Candida dubliniensis*: dark green.[10-89]

Statistical analysis

1. To test the association between the candidal carriage rate, candidal density, and species diversity among the HIV-positive individuals with and without HAART, Chi-square test was applied
2. To test the association between the candidal carriage rate, candidal density, and species diversity with the CD4 count ≤200 and >200 cells/mm^3, again, the Chi-square test was applied.

Results and Observations

The present study was aimed at quantification of candidal carriage rate, candidal density, and identification of different species of *Candida* in the oral cavity of HIV-positive individuals with and without HAART therapy.

The study included patients visiting the outpatient department of the institute hospital, as well as outpatients visiting private HIV clinics (direct walk-in clients of VCCTC, ART center).

To test if there was any association between normal individuals and HIV-positive individuals and the presence and absence (i.e., prevalence) of *Candida*, the contingencies are prepared as shown in the Table 1a.

To analyze if there was any association, the Chi-square test of independence of attributes was applied at 95% confidence level.

It was observed that the calculated value of Chi-square was 4.364 which was significant with $P = 0.037$, indicating that there was a positive relation between the two attributes (i.e., between normal and HIV-positive individuals).

Further, it was also verified from the percentage that among the HIV-positive individuals, 60% have shown the presence of *Candida*, while among the normal individuals, 36.66% have shown the presence of *Candida*.

The subjects who were chosen for the study, i.e., HIV-positive individuals were further divided into two groups, those who were on HAART treatment and those who were not on HAART treatment.

Table 1b shows the cross-contingency for this classification, i.e., individuals receiving HAART or not and whether *Candida* is present or absent.

To understand if there was any association between the treatment and prevalence of *Candida*, once again, Chi-square test was applied at 95% confidence level. It showed that calculated value of Chi-square was 6.944 which was significant with $P = 0.008$.

Regarding the patients who were on HAART treatment, 76.66% showed the presence of *Candida*. Analysis of patients who were not on HAART treatment revealed that 43.33% showed the prevalence of *Candida*.

Thus, it can be concluded that the prevalence of *Candida* was more in patients receiving HAART than those who were naïve to HAART therapy.

To identify the relationship between the candidal density and the presence or absence of HIV, the cross–tabulation, as shown in Table 2a, is used. This shows *Candida* density count between 1–2000 CFU's/ml and more than 2000 CFU's/ml. The Chi-square test at 95% of confidence level applied to the data showed that the calculated value of Chi-square was 4.96 with $P = 0.084$ which indicated that there was no significant relationship between the two, i.e., between the normal and HIV-positive individuals.

Of the patients who showed positive candidal carriage rate, 36 were in HIV-positive patients and 11 were in normal individuals. It was found that 41.66% of HIV-positive patients had candidal density >2000 CFU's/ml compared to 27.27% of normal individuals.

Table 1a: Correlation of candidal carriage rate between normal individuals and HIV-positive patients [Graph 1a]

Inidviduals	*Candida* species (present)	*Candida* species (absent)	Total
HIV (*n*=60)	60% (36)	40% (24)	60
Normal (*n*=30)	36.66% (11)	63.33% (19)	30
Total (*n*=90)	52.22% (47)	47.77% (43)	90

Table 1b: Correlation of candidal carriage rate between HIV-positive patients with and without highly active antiretroviral therapy [Graph 1b]

HIV individuals	*Candida* species (present)	*Candida* species (absent)	Total
With HAART (*n*=30)	76.66% (23)	23.33% (7)	30
Without HAART (*n*=30)	43.33% (13)	56.66% (17)	30
Total (*n*=60)	60% (36)	40% (24)	60

HAART: Highly active antiretroviral therapy

Table 2a: Correlation of candidal density (colony-forming unit's/ml) between normal individuals and HIV-positive patients [Graph 2a]

Individuals	1-2000 (CFU's/ml)	>2000 (CFU's/ml)	Total
HIV	58.33% (21)	41.66% (15)	36
Normal	72.72% (08)	27.27% (03)	11
Total	29	18	47

CFU's: Colony-forming unit's

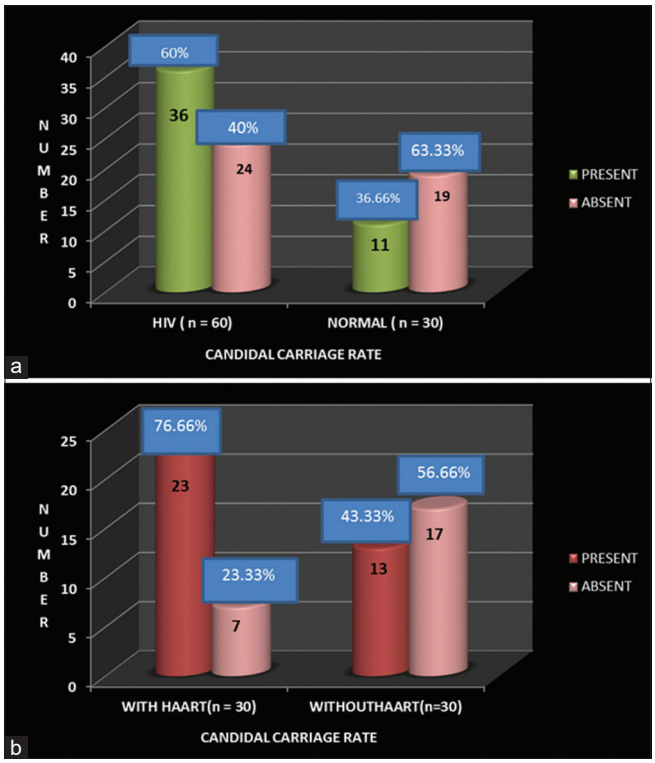
Further, we also tried to understand whether there was any association between the HAART treatment and the candidal density count, as seen in Table 2b, and applying Chi-square test at 95% confidence level. This showed that the Chi-square calculated value was 9.995 with $P = 0.007$ which was significant. Thus, it was observed that there was a relationship between the candidal density and the HAART treatment.

It was seen that 69.56% of the patients who were on HAART treatment had candidal density count between 1 and 2000 CFU's/ml, compared to 38.46% of individuals who were not on HAART.

To test if there was any association between the normal and HIV-positive individuals and the candidal diversity, the contingencies are prepared, as shown Table 3a.

To assess whether there was a significant relationship between the species diversity between the normal individuals and HIV seropositive patients, the Chi-square test at 95% of confidence level was applied to the data which showed that the calculated value of Chi-square was 6.39 with $P = 0.041$. This showed that the correlation of the species diversity between the normal individuals and HIV-positive patients was significant.

Table 3b shows the total *Candida* species isolated in the present study which included the HIV-positive individuals with and without HAART. The Chi-square test at 95% confidence level was applied to the data. The calculated value of Chi-square was 8.033 with $P = 0.18$, which indicated that there was a significant relationship between the species diversity in HAART and non-HAART HIV-seropositive individuals.



Graph 1: (a) Correlation of candidal carriage rate between normal individuals and HIV-positive patients. (b) Correlation of candidal carriage rate between HIV-positive patients with and without highly active antiretroviral therapy

Table 2b: Correlation of candidal density (colony-forming unit's/ml) between HIV-positive patients with and without highly active antiretroviral therapy [Graph 2b]

HIV individuals	1-2000 (CFU's/ml)	>2000 (CFU's/ml)	Total
With HAART	69.56% (16)	30.43% (07)	23
Without HAART	38.46% (05)	61.53% (08)	13
Total	21	15	36

HAART: Highly active antiretroviral therapy, CFU's: Colony-forming unit's

Table 3a: Correlation of candidal species diversity between normal individuals and HIV-positive patients [Graph 3a]

Individuals	Albicans	Nonalbicans	Total
HIV	80.55% (29)	19.44% (07)	36
Normal	100% (11)	0% (0)	11
Total	40	7	47

Table 3b: Correlation of Candidal species diversity between HIV-positive patients with and without highly active antiretroviral therapy [Graph 3b]

HIV individuals	Albicans	Nonalbicans	Total
With HAART	78.26% (18)	21.73% (5)	23
Without HAART	92.30% (12)	7.69% (1)	13
Total	30	6	36

HAART: Highly active antiretroviral therapy

Table 4a: Correlation between CD4 count (≤200, >200 cells/mm^3) in HIV-positive patients and candidal carriage rate [Graph 4a]

CD4 count (cells/mm^3)	*Candida* species (present)	*Candida* species (absent)	Total
≤200	65.51% (19)	34.48% (10)	29
>200	54.83% (17)	45.16% (14)	31
Total	36	24	60

Table 4b: Correlation between CD4 count (≤200, >200 cells/mm^3) in HIV-positive patients and candidal density (colony-forming unit's/ml) [Graph 4b]

CD4 count (cells/mm^3)	Candidal density (CFU's/ml)		Total
	1-2000 (CFU's/ml)	>2000 (CFU's/ml)	
≤200	52.63% (10)	47.36% (9)	19
>200	64.70% (11)	35.29% (6)	17
Total	21	15	36

CFU's: Colony-forming unit's

To know the association between the prevalence of *Candida* and the CD4 count, Chi-square test was applied at 95% confidence level. The standard classification of CD4 count ≤200 and >200 cells/mm^3 was used for HIV-positive individuals under the study. The calculated value of Chi-square was 0.712 with $P = 0.399$ which showed no significant relation between the prevalence of *Candida* carriage rate and CD4 count of HIV-positive individuals.

Chi-square was applied at 95% confidence level to check whether there was any association between CD4 count and candidal density. The calculated value of Chi-square was 1.249 with $P = 0.536$ which was not significant. Thus, it can be stated that there was no relation between CD4 count and candidal density of the individuals.

To know the relationship between CD4 count and candidal diversity, the Chi-square test was applied at 95% of confidence level. The calculated value of Chi-square was 0.066 with $P = 0.799$. The results indicated that there was no significant relationship between CD4 count and candidal diversity [Table 4a-c and Graphs 1-4].

Table 4c: Correlation between CD4 count (≤200, >200 cells/mm^3) in HIV-positive patients and candidal species diversity [Graph 4c]

CD4 count (cells/mm^3)	Candidal species diversity		Total
	Albicans	Nonalbicans	
≤200	78.94% (15)	21.05% (4)	19
>200	82.35% (14)	17.64% (3)	17
Total	29	7	36

Conclusion

HIV-positive individuals undergoing HAART therapy showed higher candidal carriage rate and lower candidal density than the non-HAART group.

Candidal density is a more valuable marker in predicting the development of OC. Hence, we conclude that HAART definitely has a role in preventing HIV-infected seropositive individuals from developing overt candidiasis. Furthermore, the emergence of increased number of NCAC species in individuals undergoing HAART suggests one to explore the dynamics of HAART action on *C. albicans* species in more detail and with different parameters.

Further studies should be conducted to gain insight into the effect of HAART on the albicans and nonalbicans species and the resistance to it at the molecular level. Furthermore, no significant association was found between OPC and CD4 count in our study, and hence, HIV viral load should be taken in consideration as a parameter.

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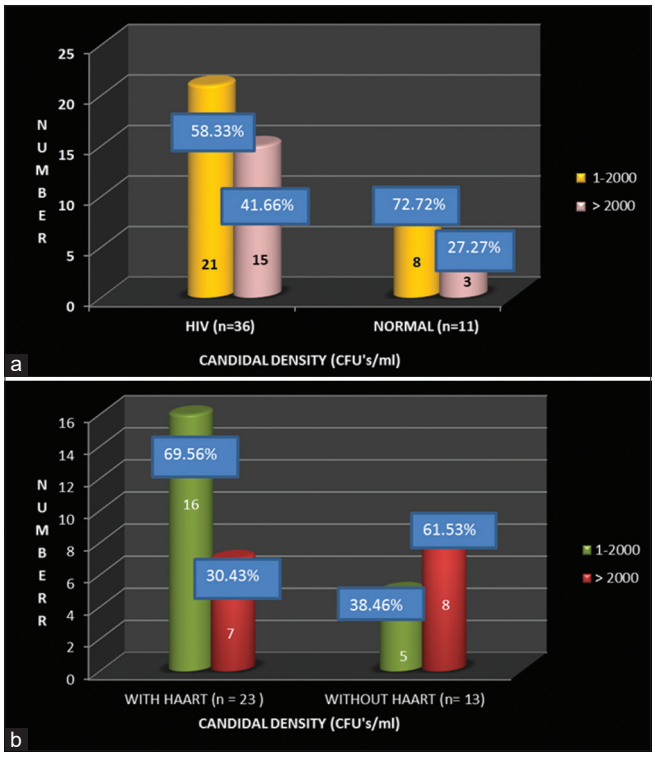
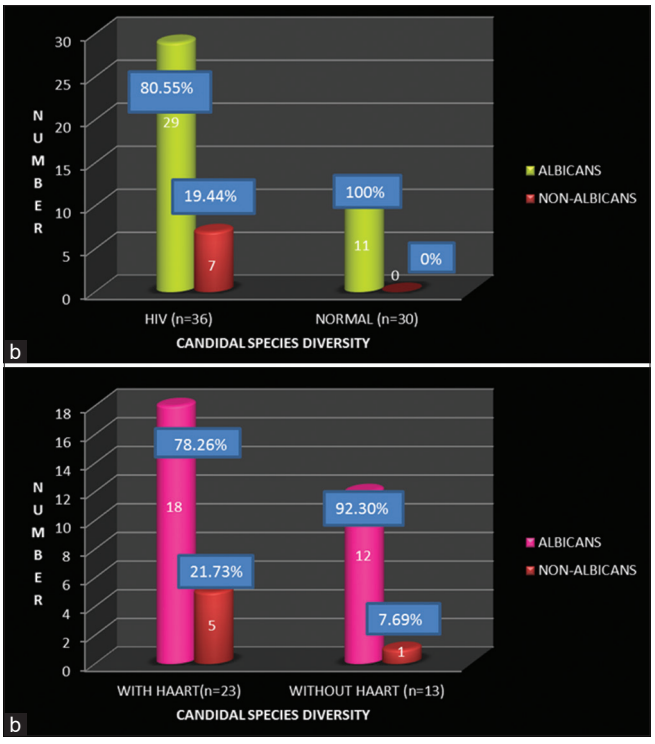
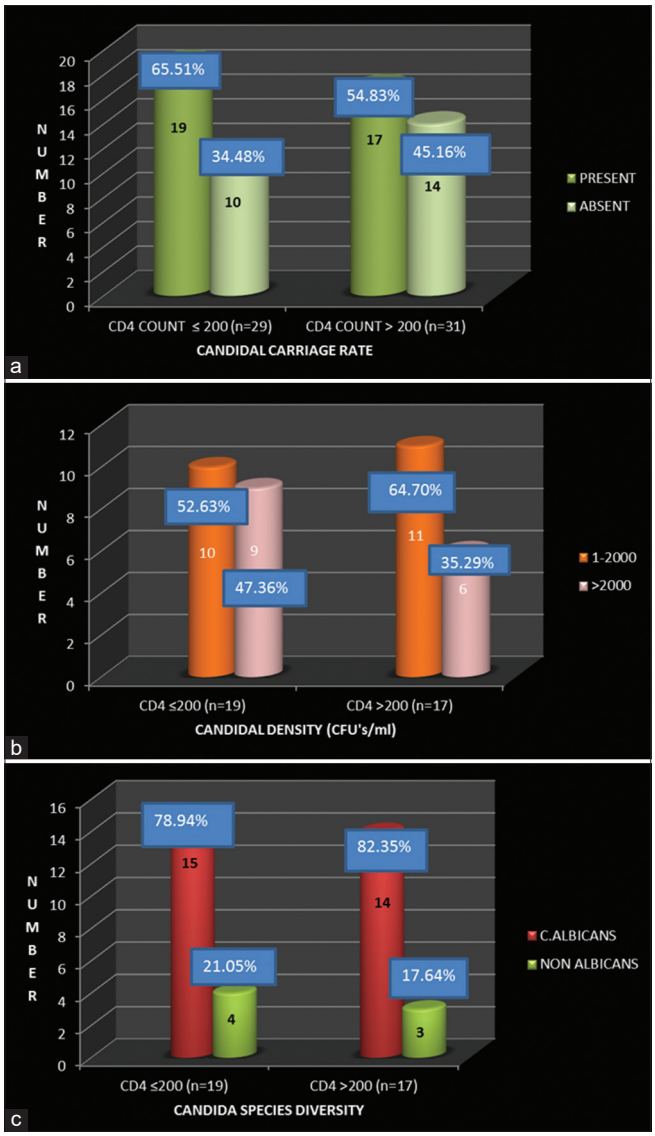
Nil.



Graph 2: (a) Correlation of candidal density (CFU's/ml) between normal individuals and HIV-positice patients. (b) Correlation of candidal density (CFU's/ml) between HIV-positive patients with and without highly active antiretroviral therapy



Graph 3: (a) Correlation of candidal species diversity between normal individuals and HIV-positive patients. (b) correlation of candidal species diversity between HIV +ve patients with and without highly active antiretroviral therapy



Graph 4: (a) Correlation between CD4 count (≤200, >200 cells/mm^3) in HIV-positive patients and candidal carriage rate. (b) Correlation between CD4 count (≤200, >200) in HIV-positive patients and candidal density (CFU's/ml). (c) Correlation between CD4 count (≤200, >200 cells/mm^3) in HIV-positive patients and candidal species diversity

Conflicts of interest

There are no conflicts of interest.

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