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Isolation and Speciation of Candida Species from Oral Thrush in HIV Seropositive Individuals from Tertiary Care Hospital

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A B S T R A C T

Infection due to Candida species are increasing in recent decades, probable reason being increase in incidence of immunosuppression, significant risk being low absolute CD4+ T lymphocyte count. Increase in frequency of non *Candida albicans* species has been observed. Hence the present study was carried out to study the incidence of *Candida* species among HIV positive patient**.** Prospective study was carried on 100 HIV seropositive individuals presenting with oral thrush. Identification of Candida species was done using KOH mount, Gram's stain and inoculated on Saboraud dextrose agar, CHROM agar and various other tests were carried out.CD4+ T cell count was done. the common age group affected was 16-45 years, with slight male preponderance. Most of the case fall either in stage 3 or stage 4. *C.albicans* was the predominant pathogen isolated accounting for nearly 52%, among the non albicans group the most common isolates were *C.dubliensis* (24%), *C.tropicalis* (15%), *C.krusei* (5%) and *C.parapsilosis* (4%). Nearly 70% of the patients had a CD4+ T-cell count of less than 200cells/µL. Candida is most common opportunistic fungal pathogen seen in HIV seropositive individual, when the CD+4 count is considerably low. Though, *C.albicans* most common pathogen isolated, there is also a significant increase in non *Candida albicans* species.

Introduction

Candida is a Gram positive polymorphic yeast like fungi that produces pseudohyphae both in culture and in tissues and exudates.[1] Candidiasis is the commonest fungal disease found in humans, affecting mucosa, skin, nails and internal organs. It is found mainly as secondary infection with some underlying immunocompromised condition.[2] In the current few years, there is a increasing evidence of candidial infections, main reason being immunosuppression due to HIV or other condition like steroid use, injudicious use of broad spectrum antibiotics and organ transplantation.[3] Although, *Candida albicans* being the most commonest cause of 60-80% of candidiasis, current statistics shows increase in the emergence of non albicans species like *C.glabarata, C.tropicalis,*

C.parapsilosis which account for nearly 30% of nosocomial infections[4]

Oropharyngeal candidiasis occurs when CD4 count is 200-500 cell/mm^3. [5]It may occur as angular chelitis, pseudomembranous/flat erythematous candidiasis or esophageal candidiasis.[6]Decreased CD4+ T- lymphocytes is an important risk factor for oropharyngeal candidiasis[7]. Oral candidiasis is a significant source of morbidity, as it can cause chronic pain or discomfort upon mastication, limiting nutrition intake in the elderly or immunodeficient patients. Hence the present study was carried out to find out the incidence of candida species from HIV affected individuals and to speciate the isolates.

Materials and Methods

The study was conducted on 100 patients attending to ART Centre after being tested reactive in ICTC, presenting with clinical suspicion of oral candidiasis. Non HIV patients with oral thrush was excluded from the study.

Two sterile cotton swabs moistened with saline water were used to collect the sample Swabs kept in sterile container with cotton plugs and transported immediately to microbiology laboratory. One swab is used for direct examination in KOH mount and Gram stain Second swab is inoculated on Saboraud's dextrose agar and chocolate agar. The following test were used to identify *Candida* species – Grams stain, KOH mount, germ tube test, growth on Saborauds dextrose agar, chocolate agar, cornmeal agar, CHROM agar, biochemical test like fermentation and assimilation tests. Culture media detects *Candida* species from 24-48 hours. Growth on the CHROM agar was noted with in 24 hrs in most of the cases, although few isolates required 48 hrs. Colour of the colonies was noted down and the species was interpreted based on the manufacturer's instructions.

Results and Discussion

A total of 100 patients attending to ART centre tested positive for HIV by all 3 tests as per NACO guidelines with signs and symptoms of oral candidiasis were included in the study Out of 100 samples, *Candida* species was isolated from 100 samples, Male preponderance was noted, the prevalence rate being 59% among males and 41% among females.57% patients belonged to stage 4, 34% patients fall in stage 3 (WHO clinical staging of HIV).

Gram stain and KOH examination were able to detect the budding yeast like cells in 64 and 78% respectively. Germ tube test was carried out in fresh human serum at 37^{0}C for 2hours. All species of *C.albicans* and *C.dublienesis* were positive for germ tube test. On inoculation into Corn meal agar, the characteristic arrangement of pseudohyphae, blastospores and chlamydospores were identified to speciate the Candida species.

*C.albicans*is the most frequently isolated species, accounting for 52%, followed by *C.dubliensis* (24%), *C.tropicalis* (15%), *C.krusei* (5%) and *C.parapsilosis*(4%).

Though *C. albicans* is the most frequently isolated organism, there is increase in the emergence of non albicans *Candida* species.

The species were identified based on the colony morphology on CHROM agar and assimilation and fermentation tests. 78% of subjects had CD4+ T cell count less than 200, while 22% of subjects had CD4+T cell count >200.

In the present study, there was higher preponderance among males, compared to females. The results were similar in a study conducted by Jayachandran, *et al.,*[8] and Shymala *et al.,*[9] which showed a higher

preponderance among males. 16- 45 age group was the most frequently affected, which was consistent with the finding reported from Vargas KG *et al.*,[10]

Table.1 Age Distribution

Age Group (Years)	No of Cases	Percentage %
0-15	0	0
16-30	31	31
31-45	48	48
46-60	16	16
61-75	5	5

Fig.1 Sex Distribution

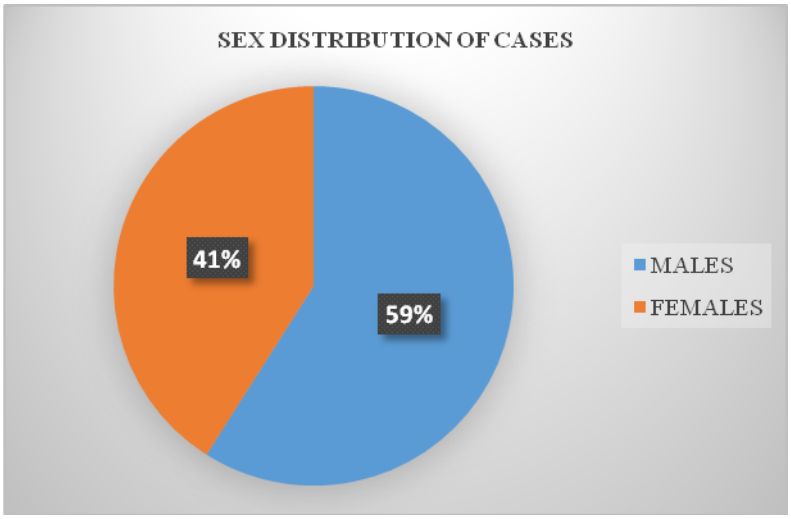


Fig.2 Species Distribution

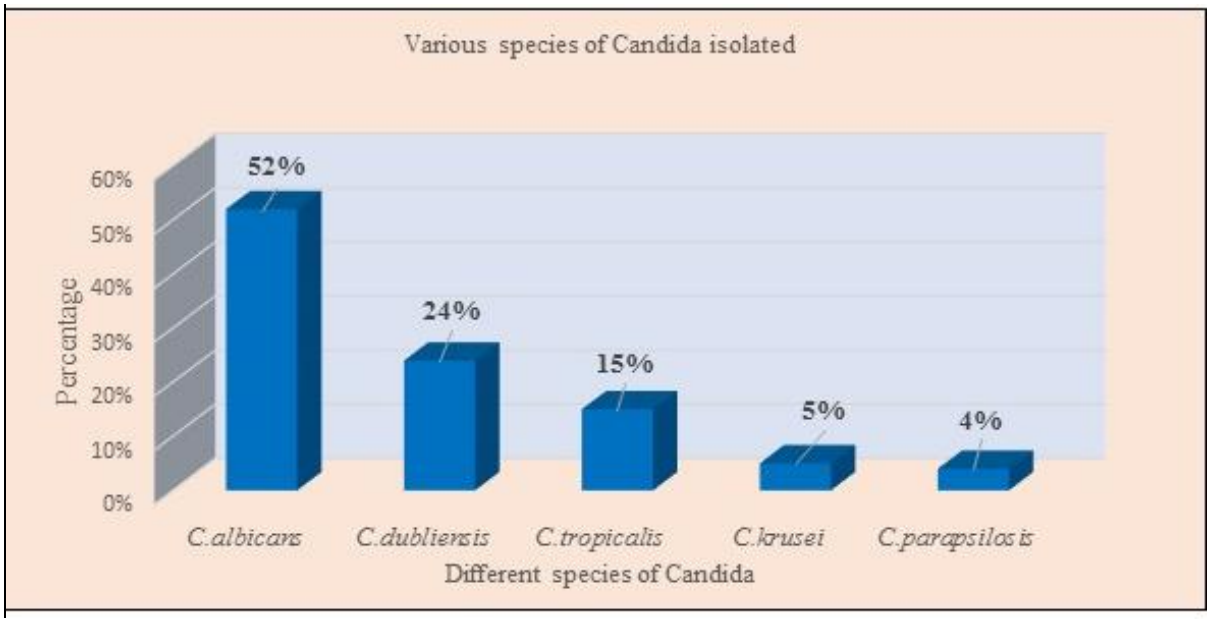
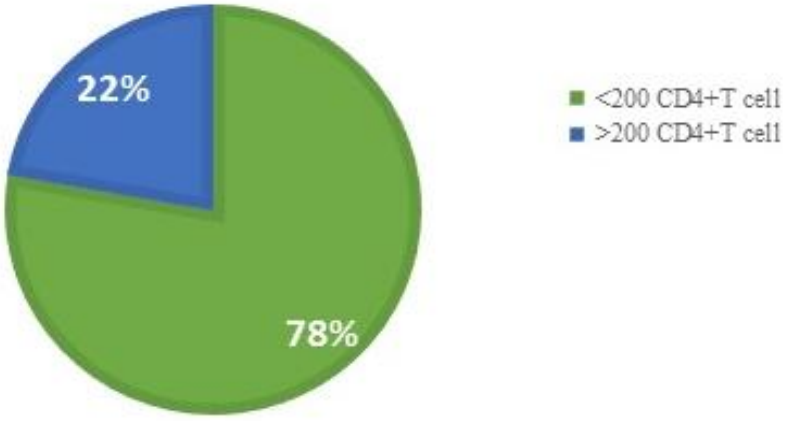


Fig.3 Distribution of Samples according to Cd4+ T Cell Count



In this study most of cases fall into stage 4 category which was similar to study by S.R. More *et al.,* .[11] The sensitivity of Gram Stain and KOH examination was 64% and 78%, similar results was noted in a study by Nadagir *et al.,*[12] The sensitivity of SDA is 100% which was similar to a study by Schmidt AM *et al.,*[13] In the present study all isolates of *C.albicans* and *C.dubliensis* were positive for germ tube test. There were no false positives, results being consistent with Singh. K *et al.,*[14].

In this study, 52% of cases were *C.albicans*, while non *Candida albicans* species were isolated 48% whereas in study by Vargas LOS *et al.,* [15] *Candida albicans* isolated was 60.7% and *Non-Candida albicans* was 33.3%. In study by S R More *et al.,*[11], *Candida albicans* isolated was 60.3% and *Non-Candida albicans* was 39.33%.Among non *Candida albicans*, *C.dubliensis* was most commonly encountered (24%), which is similar to study by More. S. R[11] *et al.,* which had 25.3% of *C.dubliensis*. In this study 15% were *C.tropicalis,* followed by *C.krusei 5%* and *C.paraspilosis 4%.* Study by Sanjay Kumar *et al.,* [16] showed the prevalence of C.tropicalis around 2% while C.krusei was 12%. In a study by Jayachandran *et al.,*[8] 56.45% of their isolates was *C.albicans* and 19.3% was *C.tropicalis*. in a study by Nadagir *et al.,*[12]*C.albicans* was reported in 66.6% of the isolates and 33.3% were non *candida albicans* species of which 48.9% was *C.dubliensis,* 20% *C.krusei,* 11% *C.parapsilosis*, 8.9% *C.tropicalis* and 4.9% *C.guillermondii*. 78% patients had CD4+ count < 200, similar to study by Arora. U *et al.,* [17] were 76.66% patients had CD4+ count < 200. Though *C.albicans* is the commonest species isolated, there is increase in the emergence of non albicans Candida species.

Candidiasis is common manifestation when there is an underlying immunosuppression. HIV being an immunosuppressed state, favors Candidial infection especially when the patient in last stage of disease manifestation or when the CD4+ T cell count is less than 200 cell/cu.mm. Prompt clinical and laboratory diagnosis has to be carried out to initiate the treatment as early as possible, to prevent the complications. With increase in non albicans Candida species, there is a necessity to speciate the Candida to species level as few species are intrinsically resistant to azoles.

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