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Oral Colonization of Candidia sps among People Living with HIV/AIDS on Anti Retroviral Therapy

Dr. Mohammed Shahid[1], Dr. Nagasrilatha Bathala[2]

Abstract: ***Background: The major important dreadful disease of the 21st century is AIDS. The prevalence is being increased globally every year and became vulnerable to the public health. As it affects immune system, people living with HIV/AIDS placed at an increased risk of a variety of opportunistic infections. Among these, oral candidiasis is the most common HIV related oral lesion and most patients infected with a strain originally present as a commensal of the oral cavity. The rate of Candida infection is inversely related to the CD4 counts of the patient. Early diagnosis of oral Candidiasis and initiation of HAART with restoration of immune status will help in improving the general well - being of the PLWHA. Objectives: 1. To determine the prevalence of oral colonization with Candidia sps, 2. speciation of Candida isolates.3. Correlation with the immune status (CD4 count) of PLWHA. Materials & Methods: A cohort prospective study was conducted among 147 Individuals on ART. Basic demographic data, CD4 count and two oral swabs were collected from each participant. One swab was subjected to Gram's staining and another one was inoculated on SDA. All the yeast isolates were speciated by germ tube test and growth on CHROM agar. The results were entered in excel sheet and analyzed. Results: Most of the participants were between 41 - 50 years (52/147). Out of 147, growth was obtained in 39 samples (26.5%). More Candida isolates were from the 41 - 50 years (35.8%). The most common Candida isolate was Candida albicans (61.53%) followed by Candida tropicalis (17.94%).46.15% of PLHA with candida colonization had CD4 count > 500 cells/mm3. Only 15.38% of PLHA with candida colonization had < 200 cells/mm3. Conclusions: As the trends of infection with Candida sps is shifting towards nonalbicans, speciation of Candida should be done as a routine diagnostic method. Irrespective of CD4 count, periodical screening for subclinical colonization with Candida sps can be recommended for prophylactic administration with antifungal drugs which can not only lead to prevention of clinical manifestations but also can minimize morbid conditions caused by these organisms.***

Keywords: HIV/AIDS, Oral colonization, Candida sps, CD_4 count

1. Introduction

The major important dreadful disease of the 21st century is AIDS (acquired immune deficiency syndrome) which is caused by Human Immunodeficiency Virus (HIV). The prevalence is being increased globallyevery yearand became vulnerable to the public health[123]. As it affects immune system, people living with HIV/AIDS (PLWHA) are placed at an increased risk of a wide variety of opportunistic infections[4]. These opportunistic infections enhance the risk of mortality and decrease the quality of life and life expectancy[5, 6]. It has been estimated that 60% to 90% of PLWHA will present with at least one oral manifestation[4]. Among these oral candidiasis is the most common HIV related oral lesion and most patients infected with a strain originally present as a commensal of the oral cavity [7, 8]. Candida albicans represents the most common causative agent of oral candidiasis; nonetheless, over the recent years, other species of Candida such as Candida glabrata, Candida krusei, Candida tropicalis, Candida parapsilosis and Candida dubliniensis, are often implicated and have been emerging with varying pathogenicity [8, 9]. The low absolute CD4+ Tlymphocyte count has traditionally been cited as the greatest risk factor for the development of oropharyngeal candidiasis and the current guidelines suggest that there will be increased risk, once CD4+ T lymphocyte counts fall below200 cells/ µl[10]. During the course of HIV infection, the rate of Candida infection is inversely related to the CD4 counts of the patient which in turn depends on the use of Anti - retroviral treatment[11]. Early diagnosis of oral Candidiasis and initiation of HAART with restoration of immune status will help in improving the general well - being of the PLWHA[12].

With regards to the above, the present study was conducted to determine the prevalence of oral colonization with Candidia sps, speciation of Candida isolates and correlation with the immune status (CD4 count) of PLWHA in a tertiary care hospital, located in South India.

2. Materials& Methods

A cohort prospective study was conducted at department of Microbiology, GMC/GGH, Kadapa from June - 2023 to August 2023.147 Individuals who are on ART were included in this study. After taking consent from the participants, basic demographic data, CD4 count and two oral swabs were collected from each participant.

One swab was subjected to Gram's staining and another one was inoculated on SDA and Blood agar. The plates were read after overnight incubation at 37°C. All the suspected yeast colonies were confirmed by Gram staining[13]. All the yeast isolates were further speciated by germ tube test and growth on CHROM agar[14]. All the samples were processed as per the standard operative procedures. The results of the study were entered in excel sheet and analyzed.

The present study was approved by the Institutional Ethics Committee.

3. Results

Most of the participants were between 41 - 50 years (52/147) followed by 31 - 40 (42/147) age group with mean age of 42.15 and median 43 years as shown in Table 1. Out of 147, growth was obtained in 39 samples (26.5%) and almost equally from both genders as shown in table 2. No Candida sps was isolated in the age group of < 10 years and > 60 years. More Candida isolates were from the 41 - 50 years (35.8%) followed by 31 - 40 years (33.33%) with mean age of 42.6 and median 44.5 years. Candida sps were isolated

almost equally from both genders with slight difference. (Male - 51.28%; Female - 48.72%). Range of PLHA with Candida sps colonization was 12 - 57 years as shown in table 3. The most common Candida isolate was Candida albicans (61.53%) followed by Candida tropicalis (17.94%) as shown in table 4.46.15% of PLHA with candida colonization had CD4 count > 500 cells/$mm^{3.}$ Only 15.38% of PLHA with candida colonization had CD4 count < 200 cells/$mm^{3.}$ The mean and median CD4 count among these participants were 571 &532 cells/mm^3 respectively as shown in table 5.

Table 1: Age wise distribution of participants

Age group	Male	Female	Total
<10	1 (0.6%)	1 (0.6%)	2 (1.28%)
11 - 20	3 (2.04%)	1 (0.6%)	4 (2.72%)
21 - 30	9 (6.12%)	8 (5.44%)	17 (11.56%)
31 - 40	25 (17%)	17 (11.56%)	42 (29%)
41 - 50	26 (17.68%)	26 (17.68%)	52 (35.37%)
51 - 60	16 (10.88%)	10 (6.80%)	26 (17.6%)
>60	3 (2.04%)	1 (0.6%)	4 (2.72%)
Total	83 (56.4%)	64 (43.6%)	147 (100%)
MEAN	42.52	41.69	42.15
MEDIAN	43	43	43
MIN	9	10	9
MAX	70	66	70

Table 2: Results of growth on culture media

	Growth	No growth	Total
Male	20 (13.6%)	63 (42.9%)	83 (56.5%)
Female	19 (12.9%)	45 (30.6%)	64 (43.5%)
Total	39 (16%)	108 (73.4%)	147 (100%)

Table 3: Age and gender vise distribution of Candida isolates

Age group	Male	Female	Total
<10	0	0	0
11 - 20	1 (2.56%)	0	1 (2.56%)
21 - 30	1 (2.56%)	3 (7.69%)	4 (10.25%)
31 - 40	7 (17.9%)	6 (15.38%)	13 (33.33%)
41 - 50	7 (17.9%)	7 (17.9%)	14 (35.8%)
51 - 60	4 (10.25%)	3 (7.69%)	7 (17.9%)
>60	0	0	0
Total	20 (51.28%)	19 (48.72%)	39 (100%)
MEAN	42.2	42.26	42.60
MEDIAN	44.5	42	44.5
MIN	12	30	12
MAX	54	57	57

Table 4: Gender vise of distribution of Candida sps

Candida species	Male	Female	Total
C. albicans	13 (33.33%)	11 (28.20%)	24 (61.53%)
C. tropicalis	2 (5.12%)	5 (12.82%)	7 (17.94%)
C. krusei	3 (7.69%)	2 (5.12%)	5 (12.82%)
C. glabrata	2 (5.12%)	1 (2.56%)	3 (7.69%)
Total	20 (51.28%)	19 (48.72%)	39 (100%)

Table 5: CD4 cell count among PLHA with candida colonisation

Age group	<200 cell/mm^3	200 - 500 cell/mm^3	>500cell/mm^3	Total
11 - 20	0	1 (2.56%)	0	1 (2.56%)
21 - 30	2 (5.12%)	1 (2.56%)	1 (2.56%)	4 (10.24%)
31 - 40	2 (5.12%)	7 (17.94%)	4 (10.25%)	13 (33.33%)
41 - 50	1 (2.56%)	4 (10.25%)	9 (23.07%)	14 (35.89%)
51 - 60	1 (2.56%)	2 (5.12%)	4 (10.25%)	7 (17.94%)
Total	6 (15.38%)	15 (38.47%)	18 (46.15%)	39
MEAN	571			
MEDIAN	532			
MIN	35			
MAX	1368			

4. Discussion

PLHA are in immunocompromised state, hence these individuals are at higher risk for opportunistic infections. Candida species are one of the common opportunistic infections in PLHA. As Candida speciesare ubiquitous fungi these organisms can cause varied manifestations from colonization to severe disseminated disease. Oral thrush and mucocutaneous candidiasis can lodge the individual with HIV/ AIDS in severe morbid conditions. Early detection of Candida colonization is helpful to minimize the morbid conditions caused by these organisms.

In the present study, 147 PLHA were screened for Candida sps colonization. Our study for oral Candida colonization had an age distribution of 9 - 70 years comprising 56.4%of males and 43.6% of females which might be due to high prevalence of HIV/AIDS in males but the age distribution of 19 - 70 years, comprising 29.8 % males and 70.2 % of femaleswas in Yitayew. et al study[15].

The most common age group screened was 41 - 50 years (35.37%), followed by 31 - 40 years (29%) which can be explained by epidemiology of HIV/AIDS but it was 51 - 60 years in a study by Shivkumar et al[16].

Yeast growth was obtained from 16% of participants in our study. It was more in Ambe et al (42.86%) [17], Hodiwala et al (44%) [7], Goulart LS et al (51.3%) [9], Agwu et al (52.2%) [18], Alexander et al (77.2%) [4]. It might be due to selection of participants in the study, as the present study was aimed to screen for sub clinical colonization with Candida sps rather than detection of Candida sps in clinically diagnosed oropharyngeal Candidiasis among PLHA.

Our present study observed that there was no much difference between males (51.28%) and females (48.72) in whom Candida sps was colonized represents that gender factor could not influence colonization of Candida sps, though some studies showed that Candida sps were isolated more in males (Suryana et al - males: 65.4%; [19]Mohammed et al – males: 59%[20]). The age group most commonly with Candida colonization was 31 - 50 years (69.13%) with mean

age 42.6 years in our study and it was almost same in a study by Sunyana et al[19] (common age group: 31 - 50 years; mean: 40 years). In our study Candida sps were isolated in wide range of age i. e.12 - 57 years and Candida sps was not isolated from the age group < 10 years and > 60 years. It might be because of periodical screening and initiated prophylaxis in these extreme age groups.

High prevalence of C. albicans is not surprising as this species is widely recognized as the most pathogenic yeast species and similar results had been observed in previous studies also.

In the present study C. albicans (61.53%) was isolated more than non albicans Candida sps (38.47%). Almost all studies showed the same results – Berberi et al: 73 %[21]; Nadagir et al.: 90.66 %[22]; Mohammed et al: 74%[20]; Ambe et al 60.2%[17]; Goulart LS et al[9]: 80%; Agwu et al[18]: 87%; Hodiwala et al: 72.7%[7;] Alexander et al: 50%[4]. Among the non albicans Candida sps, C. tropicalis (17.94%), C. krusei (12.82%), C. glabrata (7.69%) were the common species in our study. Though the common isolated non albicans Candida sps were same with different percentage of isolation in other studies. The higher detection rate of non - albicans Candida species in our study might be due to the fact that these species were previously misidentified due to its phenotypic resemblance to C. albicans and it is now being increasingly recognized with the availability of diagnostic methods. The proportion of Candida infections caused by C. albicans in PLHA has shifted towards the nonalbicans Candida species. Non - candida species tend to be less susceptible to antifungal agents and this has accounted for their emergence as a significant pathogen. 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 and check the emergence of antifungal resistant strains[4].

In the present study Candida sps were isolated more from PLHA of CD4 count > 500 cells/ mm^3 than < 500 cells/$mm^3$with the mean 571 cells/ mm3. But in other studies it was different in a study by Hodiwala et al[7] (> 550 cells - 4.5%). Colonization with Candida sps in PLHA with CD4 count < 200 cells/mm3 was 15.38% in our study which was completely different with studies by Alexander et al (89.71%) [4]; Hodiwala et al (59%) [7], as discussed earlier this might be mostly due to involved participants, periodical screening, prophylactic administration of antifungal drugs. This also reinforces the World Health Organization (WHO) recommendations to initiate treatment in adults living with HIV when their CD4+ cell counts decreased to 500 cells/mm3 or less in order to maximize the drug benefits and prevent Candida related morbidity and reduced fungal burden in People Living with HIV/AIDS (PLWHA) [4].

We strongly opined that irrespective of CD4 count, periodical screening for subclinical colonization with Candida sps can be recommended for prophylactic administration with antifungal drugs which can not only lead to prevention of evident clinical manifestations but also can minimize morbid conditions caused by these organisms. Further it can improve quality of life and life expectancy of PLHA. As the trends of infection with Candida sps is shifting towards nonalbicans, speciation of Candida should be done as a routine diagnostic method.

5. Limitations

1) Sample size is less
2) Not correlated with HIV viral load
3) Antifungal susceptibility not performed

Future studies in this region should include a larger sample size to substantiate the results, attempts to determine the susceptibility of isolates to the antifungals and accurate record of antifungal usage by patients. It would also be informative to compare the species distribution present in the oral cavities of non - HIV - infected individuals in this region.

Conflict of interest: nil

Acknowledgement: nil

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