A Comparative Evaluation of Oral Candida Carriage in HIV-Infected individuals and HIV Seronegative Healthy individuals in North Karnataka

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ABSTRACT:
Aim: Candida is one of the most common opportunistic fungi in HIV/AIDS cases. A detailed knowledge about oral candida carriage in HIV seropositive individuals is of paramount importance in the treatment of Oral Candidiasis (OC). In the present work, our aim was to determine and compare the candida carriage rate both in HIV infected and HIV non-infected healthy individuals along with knowing the species diversity of candida in HIV infected with OC and without OC and HIV non-infected healthy individuals. Methods: A total of 274 HIV seropositive individuals formed the test group. Test population was further grouped as Group 1 (HIV seropositive subjects with OC) comprising of 112 cases and Group 2 (HIV seropositive subjects without OC) comprising of 162 cases. The control group included 260 HIV seronegative healthy individuals. Concentrated oral rinse method was used to collect the specimen. Samples were inoculated on Sabouraud’s Dextrose Agar (SDA) and candida isolates were speciated by standard techniques. Statistical analysis of the data was done using Chi-square test and unpaired ‘t’ test methods. Results: HIV infected individuals with OC showed highest rate of candida carriage (97.32%). Rate of candida carriage in HIV seropositive individuals without OC (53.70%) was significantly more (p<0.001) compared to HIV seronegative healthy individuals (33.07%). In all the groups Candida albicans was the most commonly isolated species (73.48%). Conclusions: Increase in the oral candida carriage in HIV seropositive individuals may be due to immunosuppression. A few NAC spp. show high degree of resistance to commonly used antifungal. Therefore it is essential to speciate candida isolates from HIV infected individuals.

KEY-WORDS: Oral candida carriage, HIV infection, C. albicans, Oral Candidiasis.

1.INTRODUCTION
Candida is a commensal of the gastrointestinal tract and oral mucosa is the most frequent site of colonization. Colonization by this organism can lead to clinically evident infections in the presence of various predisposing factors [1-3].

Oral candidiasis (OC) is one of the most common infections seen in HIV infected patients. Most of the clinical infections are endogenously derived from colonizing candida in oral mucosa. Thus colonization of the oral cavity with candida is important in the subsequent culmination into oral or esophageal candidiasis [1, 4, 5]. It is observed that subjects are usually persistently colonized with a single strain, despite of antifungal treatment [6, 7]. Studies have shown that the rate of oral candida carriage increases with advancing HIV infection. It is also reported that CD4+ lymphocyte counts below 200 cells/mm³, is a risk factor for development of OC in HIV infected individuals [8-10]. Though several species of candida can colonize the oral cavity, Candida albicans is the most commonly isolated species in both healthy and HIV infected individuals [8, 9, 11]. Wide spread use of fluconazole has led to, increased occurrence of resistant strains of C. albicans in various specimens and increased prevalence of Non Albicans Candida (NAC) species especially in HIV infected individuals [12-17].

Though studies on oral candida carriage in HIV seropositive individuals are available from India, studies on asymptomatic oral candida carriage in HIV infected individuals
are scanty [13, 14, 16, and 17]. This is probably the first report on oral candida carriage in HIV infected individuals from North Karnataka. OC is observed to be a serious problem in HIV positive patients from this area [13]. A need, therefore, was felt to know the colonization of candida and species distribution in HIV infected individuals.

2.MATERIALS AND METHODS

2.1.Study population and data collection -
The Test Group comprised of 274 HIV-infected subjects and the control group consisted 260, age and sex matched healthy HIV seronegative subjects. HIV sero status of the individuals was tested according to the NACO guidelines using three rapid tests based on different principles (Tridot Biomed Industries, COMB Elisa Span Diagnostic Ltd, HIV Capillus, Trinity Biotech Plc.). The individuals were clinically examined for the presence of OC.
The test group was further subdivided into
- Group 1: HIV seropositive subjects with clinically proven OC (n= 112).
- Group 2: HIV infected group without OC (n=162).
Individuals with history of antifungal or antibiotics treatment within three months prior to the study and with diabetes mellitus were excluded from the study.

2.2.Collection of samples:
Samples were collected after obtaining informed consent from the subjects. Concentrated oral rinse method was used to collect the specimen which gives the semi quantitative measure of the oral candida carriage [18]. In brief, the subject was instructed to rinse the mouth with 10 ml sterile Phosphate Buffer Saline (PBS, pH 7.2) for one minute and then spit in to a sterile container.

2.3.Culture and Identification:
The oral rinse specimen was immediately transported to the laboratory. Sample was centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the sediment was suspended in one ml of sterile PBS (pH - 7.2) and vortexed for one minute. With a sterile micropipette, 100 µl of this preparation was inoculated on Sabouraud’s Dextrose Agar (SDA) and was spread evenly with sterile L-spreader. The plates were incubated at 37 0C for 48 hrs. The colonies of candida were counted and colony forming units per ml (CFU/ml) for Candida spp. were calculated. The colonies were identified on the basis of Gram’s stain, germ tube test, chlamydospore formation, carbohydrate assimilation and temperature sensitivity test [19].

2.4.Statistical Analysis:
The rate of candida carriage and prevalence of Candida spp. was statistically analyzed using Chi-square test. Mean density of candida carriage in each group was statistically analyzed using unpaired ‘t’ test.

3.RESULTS

3.1.Oral candida carriage rate:
The oral candida carriage rate in all the groups is shown in Table 1. The test group showed highest candida isolation of 71.53% (196/274) as compared to control group 33.07% (86/260). In test group, Group 1 (HIV infected individuals with OC) showed 97.32% (109/112) of candida isolation. Group 2 (HIV infected individuals without OC, showed 53.70% of candida carriage. The candida isolation rate in Group 2 was significantly higher compared to control group; p value being < 0.001. Isolation of more than one (multiple) type of candida species was observed from a few samples. Out of 282 Candida positive samples,
Table 1.
Candida isolation rate in the test groups and control population

<table>
<thead>
<tr>
<th>Study Group (n)</th>
<th>Candida Carriage n (%)</th>
<th>Total candida isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test group (274)</td>
<td>196 (71.53)</td>
<td>216</td>
</tr>
<tr>
<td>Group 1 (112)</td>
<td>109 (97.32)</td>
<td>119</td>
</tr>
<tr>
<td>Group 2 (162)</td>
<td>87 (53.70)</td>
<td>97</td>
</tr>
<tr>
<td>Control (260)</td>
<td>86 (33.07)</td>
<td>97</td>
</tr>
</tbody>
</table>

Table 2.
Prevalence of NAC species in different groups of study population

<table>
<thead>
<tr>
<th>NAC SPECIES</th>
<th>Test</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. tropicalis</td>
<td>17</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>16</td>
<td>7</td>
<td>9</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>C. krusei</td>
<td>13</td>
<td>3</td>
<td>10</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>C. zeylanoides</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>25</td>
<td>38</td>
<td>20</td>
<td>83</td>
</tr>
</tbody>
</table>

Graph 1.
Prevalence of C. albicans Vs NAC species (in percentage).
29 (10.28%) samples gave multiple candida isolates. Most commonly seen combination was *C. albicans* with *C. tropicalis* (20 out of 29).

We observed less candida carriage rate in female subjects compared to male subjects in all three groups. However, the difference was not statistically significant.

### 3.2. Mean oral candida carriage density:

For each candida positive case, CFU/ml was determined. HIV infected patients without OC yielded mean candida carriage density of 9,146 (±28,536)/ml which was far lesser than the mean candida carriage density in HIV positive individuals with OC (62,931±48,180/ml).

The difference in mean oral candida carriage density between these two groups was highly significant with p value < 0.001. We have also observed that, oral candida carriage density in control population was 2,696 ± 15,999/ml, which was significantly lesser than mean candida carriage obtained from HIV infected individuals without OC (p < 0.01).

### 3.3. *C. albicans* Vs Non Albicans Candida (NAC):

*C. albicans* was the predominant species isolated from all the groups. A total of 313 candida isolates were obtained in the present study. Among them, 230 (73.48%) were *C. albicans* and 83 (26.51%) were NAC species.

### 3.4. Distribution of NAC species:

Distribution of NAC in entire study population and in individual groups is shown in Graph 2 and Table 2 respectively. A total of 83 NAC isolates were present in the study.
Eight different NAC species were encountered in the study. *C. tropicalis* was the predominant isolate, 27 out of 83 (32.53%) among NAC species. It was followed by *C. guilliermondii*, 21/83 (25.30 %) and *C. krusei*, 14/83 (16.86%).

*C. tropicalis* was 32% and 50% being predominant NAC in Group 1 and control population respectively. However in Group 2, *C. krusei* was the predominant NAC (26.31%; 10/38 of NAC isolates).

4. DISCUSSION

Oral candidiasis is the most common candida infection seen in HIV infected individuals [1, 4, 5]. The present study showed that 40.8% of the test subjects had clinically apparent OC which is in accordance with the observations of Nadiger S. D et al who reported 38.8% clinically proven OC cases in HIV positive patients from this region [13].

Oral candida carriage and its density in HIV infected subjects vary significantly in OC and asymptomatic carriage. We observed significantly high rate of candida carriage in HIV infected subjects without OC, as compared to healthy volunteers (Group 2 and control group). Our results are in agreement with other reports on candida carriage in HIV infected individuals [9, 11]. In contrast Liu X et al [12] showed that asymptomatic oral candida carriage in HIV positive group was similar to that in healthy group.

In our study the mean density of candida carriage in Group 1 was much higher compared to Group 2 (p < 0.001) and mean density of candida carriage between Group 2 and control population was significantly high (p < 0.01). Similar observations were made by Vargas and Joly [5] and Girish Kumar CP et al [17]. Vargas and Joly [5] showed that density of oral candida carriage increases in HIV infected subjects significantly during the progression from asymptomatic yeast carriage to an episode of oral thrush. It is established that diminished host defense in HIV infection which is a multifactor process including local and systemic causes that accentuates the colonization of commensal candida [20]. In our study, Group 1 (HIV infected individuals with OC); showed significantly higher rate of isolation of *C. albicans* as compared to Group 2 which is comparable with other reports [10, 11]. Though it would appear that, *C. albicans* may replace less virulent NAC species in OC, recent studies demonstrated an increasing rate of isolation of NAC species in OC cases [13,17].

There were a good number of NAC isolated in our study from both test and control groups. *C. tropicalis*, was the most predominant NAC isolate. *C. tropicalis*, *C. glabrata*, *C. krusei* were the most common NAC species found as oral colonizers in HIV seropositive individuals in previous reports [5,8-12, 15-17]. In connection to this, we would like to highlight a unique finding of our study i.e., isolation of *C. guilliermondii* as one of the predominant NAC species from both test and control subjects. This variation could be attributed to variation in geographical distribution of yeast species. It is important to note that *C. guilliermondii* shows high MIC for fluconazole, which is the common azole used for treatment of OC [14]. Predominant isolation of *C. guilliermondii*, has been reported by Xu J and Mitchell TG [2] in their report on evaluation of healthy subjects. As a few other NAC spp also show high degree of resistance to fluconazole, it is essential to speciate candida isolates from HIV infected individuals to facilitate the treatment of OC.
5. CONCLUSION

It is important to have information on the prevalence of NAC in a particular geographical area as it would help in designing treatment in the OC cases.

6. ACKNOWLEDGEMENT

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7. REFERENCES